Condition-dependent sexual selection in a wild population of the field cricket, *Gryllus campestris*

Submitted by Ian Skicko to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences,
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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……

Acknowledgements

My parents always told me that I had “a good brain in my head” and that if I put my mind to it I could achieve anything I wanted. This seems improbable, and “a good brain” is a term that is ambiguous to the point of irrelevance, but it serves to exemplify the unending support I have received from my family in everything I have set out to achieve. I am fortunate enough to have been raised by parents who have been mentors, a brother who has been a friend, cousins who have been sisters, an aunt and uncle who have been guardians, and grandparents who have been role models. My sincere thanks go to each of you for your continuous support and for making me who I am. Without you all, this thesis, and many things that have come before it, would not have been possible.

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Abstract

Condition-dependent sexual selection has the potential to align natural and sexual selection and accelerate adaptation. When the expression of a sexually selected trait is constrained by the condition of the bearer, it offers a reliable signal of quality on which females can base mate choice decisions. Individuals with highly expressed sexually selected traits are therefore expected to possess advantageous genes given the prevailing environmental conditions. Such genes can then spread by their naturally selected benefits as well as their sexually selected advantages, thereby accelerating adaptation. I investigate the effect of condition-dependent traits on mating and signalling behaviour to explore the potential for alignment between natural and sexual selection in the wild. By studying a wild population of the field cricket, *Gryllus campestris*, I explore condition-dependent sexual selection in a natural context. This avoids some limitations of laboratory studies, which may overestimate effects in the absence of natural and environmental variation. I employ a direct experimental test of the effect of condition on sexually selected traits and mating success, finding that while male acoustic signals are condition-dependent, modest increases in calling effort do not result in increased mating success. I investigate the effect of body size on mating success and find mating success to be independent of body size. I explore the possibility of condition- and context-mediated flexibility in mate-searching tactics, finding that while population density influences tactic choice, individual condition is unlikely to predict which tactic a male will adopt. Finally, I consider the role of female condition in sexual selection and find that mating latency in females is not condition-dependent, but that mating history has an important effect on female choosiness.

Table of Contents

List of Figures and Tables

Chapter One

General Introduction and Methodology

Natural and sexual selection

While it received less publicity than natural selection, Darwin's theory of sexual selection was certainly more controversial at the time of publication, and was resisted even among Darwin's supporters (Andersson, 1994, Gayon, 2010, Parker, 1979). Darwin developed this theory as a way of explaining elaborate and seemingly costly traits which would appear to defy his theory of natural selection. Sexual selection received so little support, being broadly dismissed by some prominent naturalists of the time, that it was largely overlooked until much later (Andersson, 1994, Parker, 1979). In the last few decades however, the theory of sexual selection has become one of the most investigated topics in evolutionary biology.

It is often debated as to how strong a distinction there is between natural and sexual selection. While Darwin apparently considered them to be quite distinct processes, others have argued that sexual selection should be considered as a type of natural selection (Arbuthnott and Rundle, 2014). There is merit to the arguments made on both sides, and while this thesis is not the place to continue the debate, it does serve my purpose to refer to these processes separately. I here adopt a similar definition of sexual selection as Darwin, who considered this to be "the advantage which certain individuals have over other individuals of the same sex and species solely in respect of reproduction" (Darwin, 1871) and therefore "depends not on the struggle for existence, but on the struggle between males for possession of females" (Darwin, 1871). A more accurate, modern definition of sexual selection may prefer "access to fertilisation" over "possession of females" but put simply, sexual selection can be considered as intra-specific competition for reproductive success, while natural selection is a result of variation in any other component of fitness that is not directly

related to reproductive success (Hosken and House, 2011). Many traits may be under both natural and sexual selection, but while sexual selection is not expected to increase survival, it promotes individuals which provide more offspring to the next generation.

Due to anisogamy (the difference in gamete size between males and females of sexually reproducing species) the costs associated with reproduction differ between the sexes (Andersson, 1994). The reproductive success of males, who produce small, inexpensive gametes, is often dependent on the number of females he mates with. On the other hand, the reproductive success of females, who produce larger, more expensive gametes, is limited by the resources she can invest in gamete production (Parker et al., 1972). While there are exceptions, this difference in reproductive investment has led to males generally being driven to compete for access to females or fertilisation opportunities, and females generally being driven to be more selective about the male or males with whom she mates (Parker et al., 1972). As such, throughout this thesis females will be regarded as the choosing sex, while males will be regarded as the competitive sex, yet it is acknowledged that this is not the case in all systems.

Sexual selection is often considered to fall into two broad categories: male-male competition and female mate choice. Male-male competition describes direct conflict among males for access to females (Andersson, 1994). This can manifest as the defending of territories which overlap female home ranges, the maintenance of harems, dominance hierarchies, or the exclusion of rivals. Female mate choice on the other hand arises when females exhibit differential preferences for potential

mates based on their perceived attractiveness of those individuals (Darwin, 1871). Females may choose to preferentially mate with the most attractive males, or invest more in the offspring of the most attractive males.

The idea that female preference should have such a fundamental effect on the success of males was met with considerable resistance when sexual selection was first described (Andersson, 1994, and refs therein). It wasn't until much later that the evolutionary mechanisms of mate choice were investigated more closely. For females, choosing to mate can be costly (Daly, 1978); perhaps due to energetic costs associated with gestation and rearing, loss of time for other activities during mating or incubation, vulnerability during incubation, or choosing to mate with one male may prevent mating with a superior male. The costs of mating must therefore be offset by the benefits that can be gained from the mate, either directly or genetically. Direct benefits are those that improve the fitness of the female in terms of her own survival or fecundity (See Kokko *et al.* 2002). Such benefits could be obtained from a male if he is able to defend the female from predators, or other courting males (who may cause male-induced harm), by being a better parent, or by defending a superior foraging territory allowing the female easier access to resources. Genetic benefits are those that the female does not experience herself, but that apply to her offspring, in terms of the genes that they will inherit from him. Genetic benefits of mate choice can therefore increase a female's fitness by improving the quality of her offspring when she preferentially mates with a high-quality male (See Kokko *et al.* 2002). Understanding how females are able to determine a male's quality and mate discriminately with the best males has been a

prominent question in evolutionary biology and forms a large part of the literature of animal signalling.

Animal Signalling

A signal is defined as a trait which has evolved to provide information to a receiver (Maynard Smith and Harper, 1988) and is not limited to mate choice; animal signals are also frequently observed in male-male competition and many other intra- and inter-specific interactions. The signal might provide information on the sender's stamina (Mowles, Cotton and Briffa, 2009), immunocompetency (Zuk, Thornhill and Ligon, 1990) or nutrition (Hill, 1990), as examples. The signal is reliable if a correlation between the signal and the underlying quality of the sender is maintained (Zahavi, 1975; Grafen 1990a, 1990b). If this was not the case, and males presented a highly exaggerated signal without investing in underlying quality, they would receive a maximal fitness benefit; however, females would risk mating with poor quality males, allowing the spread of disadvantageous traits through the population (Dawkins and Guilford, 1991). In this case, selection should not favour the use of the trait in mate choice decisions, disrupting the signalling system. Signalling systems therefore require that signals are a reliable and honest indicator of quality to remain evolutionarily stable (Grafen 1990a, 1990b).

The earliest model of mate choice operating by this mechanism is the "Handicap Principle" put forth verbally by Zahavi (1975) and later supported quantitatively by Grafen (1990a, 1990b). The handicap principle rests on the assumption that the production of the elaborate traits and displays often associated with sexually selected traits, exert a cost on the bearer under natural selection. For example, the

bigger, brighter and more iridescent a peacock's tail is, the greater his reproductive success (Petrie and Halliday, 1994, Petrie et al., 1991). Investing in the development of this trait is costly and so the tail places a handicap upon the male; and the bigger the handicap, the better the male must be to survive (Andersson, 1994). Such signals represent condition-dependent traits; a trait whereby the tolerable level of expression shows genetic covariance with the quality of the individual (Lorch et al., 2003). The intensity of a trait is therefore an honest signal of the quality of that individual, which females can then use to inform their mate choice decisions. Those males with the most attractive signals are assumed to be those most likely to have the genetic background required for success under the prevailing environmental conditions (Emlen and Oring, 1977). The male may be able to withstand having a conspicuous trait in the face of predation risk, be able to gather sufficient resources to invest in elaborate ornamentation, or effectively utilise resources to sustain high intensity displays (Rowe and Houle, 1996). Whatever the reason, the male is considered attractive because the fitness of the female is improved by capturing these advantageous genes for her offspring.

Condition-dependent traits and adaptation

An established population is usually well adapted to its environment. That is to say, individuals in the population carry genes that allow them to persist in the prevailing environmental conditions (Emlen and Oring, 1977). To be well adapted, individuals must express traits close to the naturally selected optimum as at this level they are best able to utilise the resources in their environment. In practise however, a well-adapted individual will express traits at a level that is balanced between natural and sexual selection such that fitness costs incurred from sexually selected traits are

offset by increased reproductive output (Lande, 1976). When the environment changes however, this cost-benefit structure can be disrupted leading to a net loss in fitness (Candolin and Heuschele, 2008) requiring the population to adapt and find a new optimal level of trait expression, or adaptive peak. While optimal levels of trait expression under natural selection are those which maximise survival, optimal levels under sexual selection are those that maximise an individual's fertilisation success, and how much its mates invest in their shared offspring. This can cause misalignment of natural and sexual selection such that when these processes drive trait expression to the new adaptive peak, they may drive in different directions (Arbuthnott and Rundle, 2014).

Despite conferring a reproductive benefit to the individual, sexually selected exaggeration of a trait can have a detrimental impact on the viability of the population (Kokko and Brooks, 2003) and can therefore be considered maladaptive. If maladaptive genes are promoted within the population then the rate of adaptation is slowed. However, when condition-dependent traits are involved in mate choice, or in any activity that would increase the reproductive success of the individual, they represent a potential alignment of natural and sexual selection. Trait exaggeration, as a result of sexual selection, is limited by aspects of male quality that are selected for by natural selection. Females are therefore making mate choice decisions based on capturing the best genes available in the population rather than on arbitrary characteristics (Zahavi, 1975; Grafen 1990a, 199b; Rowe and Houle 1996). Beneficial genes then spread due to their naturally selected survival benefits, as well as their sexually selected reproductive benefits. This increases the overall selective pressure on the trait allowing sexual selection to accelerate adaptation.

Ecology of *Gryllus campestris* Linnaeus (Gryllidae)

Crickets of the family Gryllidae are a popular model system in studies of sexual selection however many studies are conducted only under laboratory conditions (Table 4). Here I use a wild population of one such Gryllid species, *Gryllus campestris*, to address questions of condition-dependent sexual selection in the natural environment of this population

G. campestris, is a large, flightless cricket characterised by shiny black coloration and a yellow-orange stripe at the anterior of the dorsal side of the abdomen (see Figure 1). Males and females can be differentiated by the texture of the forewing, which is altered by the development of calling structures in males, and by the presence of a long, slender ovipositor in females (Figure 1). *G.campestris* is found throughout Central and Southern Europe, Western Asia and North Africa (Marshall and Haes, 1988). Populations are threatened at the northern edges of this range, namely in the United Kingdom, Germany, Netherlands, Denmark, and Switzerland (Hochkirch *et al*., 2007, and refs therein), however conservation efforts are proving successful in some of these regions (Hochkirch et al., 2007, Pearce-Kelly et al., 1998). *G. campestris* prefers short vegetation or bare ground with a loose chalky or sandy substrate. They are vulnerable to over-shading by vegetation and so prefer open ground (Benton, 2012)

A. A male (TT) and a female (LU) outside a burrow entrance. Note the crenulations of the male wings due to calling apparatus, and the female's ovipositor visible at the base of her the abdomen.	
B. A female (VS) mounting a male in copulation. If successful, the male will attach a spermatophore to the base of the female's ovipositor (not visible in this image).	
C. Fighting between two males. Aggressive behaviours range from, antennal fencing through to wrestling, mandible flaring and biting which can result in loss of limbs and occasionally fatalities.	

Figure 1: Images showing a male and female <u>G. campestris</u> individuals (A), mating occuring between a male and female (B), and two males fighting (C).

Individuals are largely solitary and construct blind-ended burrows in the substrate, which feature a single point of access and do not interconnect (Rolando Rodriguez-Muñoz, *pers. comms)*. Burrows provide crickets with shelter from adverse weather conditions and refuge from predators (Rodriguez-Munoz et al., 2011). Although males and females will frequently move to new burrows, both sexes are highly territorial and will attempt to defend burrows from invading individuals of the same sex (Rodriguez-Munoz et al., 2011*)*. Burrows are first created by nymphs and the diameter is increased as the cricket grows. Adult crickets will typically usurp existing burrows rather than excavate new burrows (Rolando Rodriguez-Muñoz, *pers. comms)*. Grasses, which form the basis of the *G. campestris* diet, are foraged from within the immediate vicinity of the burrow entrance (Rolando Rodriguez-Muñoz, *pers. comms)*. Crickets have also been observed to occasionally eat other small invertebrates (*pers. obs.)*. Birds and rodents are the main predator groups for *G. campestris* although predation by spiders, assassin bugs and domestic cats have also been observed (Rolando Rodriguez-Muñoz, *pers. comms)*.

The *G. campestris* mating system exhibits strong female choice and both sexes are highly promiscuous. Male field crickets can influence female mating decisions using signals encompassing chemical, tactile and auditory modalities (Loher and Dambach, 1989, Tregenza and Wedell, 1997). The production of the characteristic calling song is the primary mode by which *G. campestris* males encounter females, although an alternative searching tactic has been described in this and other species of field cricket (Cade and Cade, 1992, Hissmann, 1990). Characteristics of the calling song vary among males (Popov and Shuvalov, 1977), allowing this trait to be used by females to discriminate among

potential mates. Males produce calling song through stridulation of the harp and scraper structures of the forewing (Alexander, 1961, Bennet-Clark, 1989) to attract females to their burrow from afar. When a female is encountered, males typically produce a softer courtship song to entice the female to mate. The typical characteristics of calling and courtship song in *G. campestris* are very similar to the closely related *Gryllus bimaculatus.* Calling songs are comprised of a series of chirps, each of which typically has between three and five pulses (Green, 2008). Intervals between chirps are several times longer than the chirps themselves (Green, 2008). In contrast, courtship song involves production of a more or less continuous high frequency, buzzing-like song. Courtship song is produced by engaging the harp and scraper structures with less force, producing sound in both directions of the wing stroke; instead of in a single direction as is the case with calling song (Alexander, 1961). The temporal structure of both calls varies among males, as does the carrier frequency of the call and so male calls may represent a multicomponent signal (Scheuber et al., 2003). In other gryllid species, females have been shown to prefer males who produce a higher chirp rate (*G. lineaticeps* (Beckers and Wagner Jr, 2011, Wagner Jr, 1996, Wagner et al., 2001)) and calling bout duration (*G. integer*: (Hedrick, 1986), *G. lineaticeps* (Wagner Jr, 1996)), which represent increased energetic costs, and those with a lower carrier frequency, which may indicate body size (*G. campestris*: (Simmons and Ritchie, 1996), *G. bimaculatus:* (Simmons, 1988)). The alternative searching tactic occurs in silence while males move among burrows in search of females. While males invest a great deal in mate-searching, females may arrive at male-occupied burrows in the absence of the calling song (*pers. obs.*), and it is expected that mating is

possible in the absence of courtship song as has been shown in other field cricket species (Zuk et al., 2006).

Copulation commences when the female mounts the male allowing him to deposit a spermatophore at the base of her ovipositor. Mating is terminated when the female dismounts the male, thus females have complete control over which males to mate with, and the duration of copulation. Once attached, the spermatophore transfers sperm to the female's spermatheca until it is depleted, dislodged by subsequent copulation attempts, or the female removes it, similar to the closely related *Gryllus bimaculatus* (Simmons 1986a). A mated male and female may part shortly after mating or remain at the burrow for an extended time during which males engage in mate guarding behaviour. During this time, the male allows the mated female to enter the burrow first, while he remains at the entrance to defend against invaders and serve as a distraction to potential predators (Rodriguez-Munoz et al., 2011) thereby protecting his investment in the next generation. Repeated mating is common during this time, but males do not appear to restrict female movements (Rodriguez-Munoz et al., 2011, Rost and Honegger, 1987) nor cause physical harm (Rodriguez-Munoz et al., 2011).

G. campestris is a univoltine species and females lay eggs from the beginning of the reproductive season (approximately late April at my study site) until the end of the reproductive season. Young nymphs develop for approximately ten instars before overwintering in diapause. They become active again in early spring and undergo a further moult before emerging as adults (Benton, 2012). At my study site, between 2006 and 2016, individuals began mating on average

15.24 days post-emergence and the reproductive season typically continued until July.

While movement among borrows is common for both sexes, crickets are vulnerable to predation and adverse weather during transit. Encounter rates between individuals while moving to a new burrow are presumably very low and males have not been observed to call while away from a burrow. As crickets spend most of their time in or around their burrows, it is expected that observations made at the burrow will accurately represent typical cricket behaviour.

Working in the wild

A primary aim of biological science, and indeed science generally, is to understand how the world works. Often, logistical and practical constraints require investigations to be conducted in artificial systems, constructed so as to control all aspects of natural variation other than the phenomena of interest. This experimental approach is powerful and remains one of our greatest scientific tools, however it is not without its limitations. Laboratory experimental designs act as simplified models of natural systems which assume that an individual's behaviour under laboratory conditions predicts its behaviour in the wild. This may not always be the case as it has been shown that the context in which an individual is observed can have a drastic effect on the behaviour of that individual (Barnett, 1958; Fisher *et al.* 2015; Mineka et al., 1980; Höjesjö et al., 2002; Wright et al., 2006). Parameters, often standardised in the laboratory (such as resource availability, predation risk, population density, mate availability, and mating history), can affect individual behaviour (Cade and

Cade, 1992; Hunt et al. 2004; Jirotkul, 1999b; Lierheimer and Tinghitella, 2017; Simmons, 1986a; Tinghitella, 2014) and so studies often risk oversimplifying the situation as it would be in nature, resulting in poor estimations of the natural state. If we are to best understand the world, studies that preserve natural variation and realistic environmental conditions are necessary. The best way to achieve this is by conducting experiments in natural systems, or through intensive observation of wild populations. Advances in monitoring technology are making this possible. For example, low-cost camera traps are making it possible to observe secretive and elusive animals in their natural environment, advances in tagging and tracking technologies are making it possible to remotely monitor behaviour in wild animals, and the advent of low-cost unmanned aerial vehicles is providing unique insights into the natural world.

General Methodology

The WildCrickets Field Site

The WildCrickets field site, located in Asturias, Spain, is comprised of an approximately 5,000 m^2 meadow, bordered on three sides by road and railway (see Figure 2). A wild population of *G. campestris* has been established at this site for at least 40 years located mainly in an 800 m^2 portion of relatively flat land within the meadow (Rolando Rodriguez-Muñoz *pers. comms*). The meadow is surrounded by an area shaded by tree cover. Burrows are almost never found in shaded areas and this region may serve to minimise immigration and emigration of the cricket population from a neighbouring meadow.

Annual meadow management is consistent among years: the grass is cut short in mid-March, before nymphs are active, and between July and August, after the reproductive season. The grass is kept short outside of the field season with further mowing as necessary. Seven temperature probes are fitted throughout the meadow; three fitted above ground to monitor ambient air temperature and four below ground, housed in plastic tubes, to estimate the temperature of the cricket burrows.

Cricket burrows are detected by systematic searches of the meadow conducted each week from February until the last known cricket dies the end of the reproductive season. Every burrow is flagged with a unique number to help locate and identify it throughout the season. Burrow positions were mapped each year (Figure 3). This was done using a total station (iCon Builder i60, Leica Geosystems, Switzerland) in 2015 – 2016 and by measuring the distance from each burrow to three known positions and calculating the burrow position using trigonometry in previous years. New burrows were added to the seasonal maps as they were discovered.

Figure 2: Image showing the WildCrickets field site with cameras positioned over burrows. This photograph was taken early in the season while the grass is still short, but tracks of shorter grass used by researchers to move around the meadow are still visible.

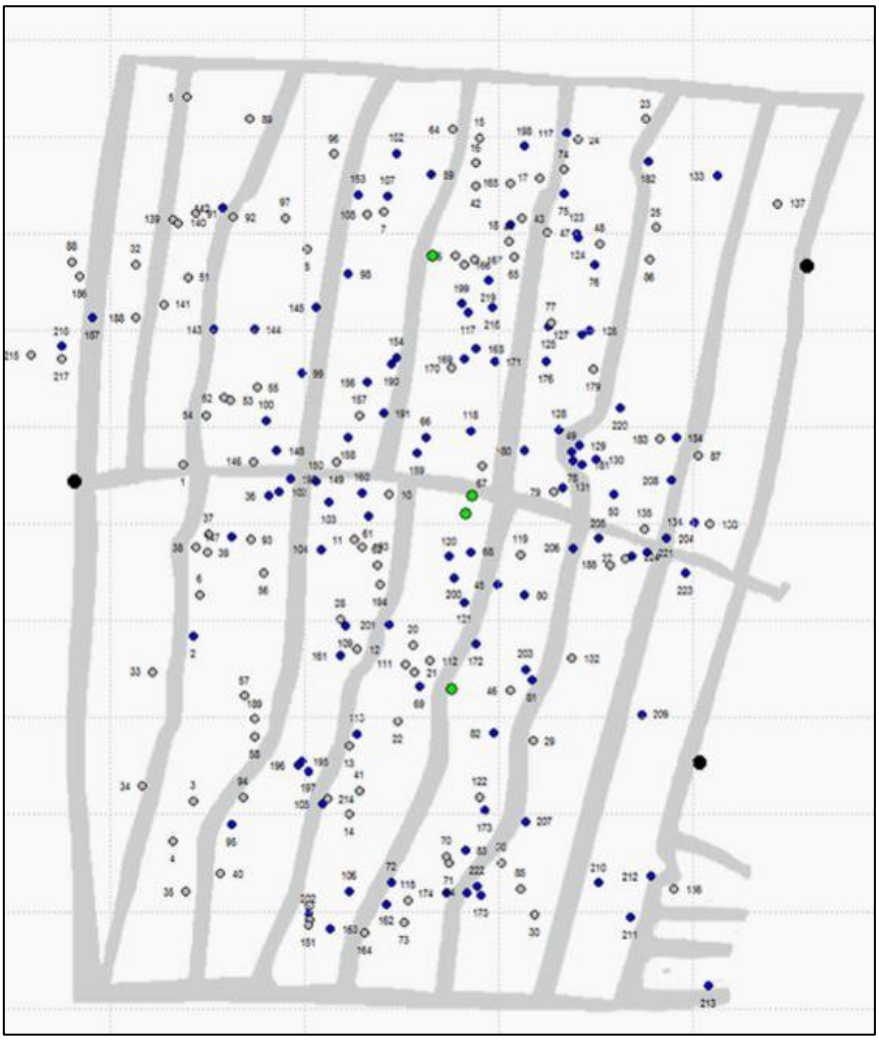

Figure 3: A map of known burrow locations made during the 2015 field season using a Leica iCon Builder i60 total station and plotted in R. Grey shaded areas represent paths used by researchers to move through the meadow without disturbing the crickets. Blue points represent burrows in active use while grey points represent abandoned burrows. Green points represent the position of temperature probes and black points represent fixed positions used to calibrate maps among years.

Up to 133 digital video cameras (VivoTek, Taiwan) with day-night capability were positioned over burrows to monitor cricket activity without interfering in natural behaviour. The number of deployable cameras increased from 64 in 2006 to the current 133 as the project expanded. These cameras ran continuously from between late March and mid-April, prior to adult emergence, throughout each reproductive season to record cricket behaviour. The cameras were connected to a bank of computers running motion-activated recording software (Diginet, Kodicom Ltd, South Korea, between 2006 and 2010; iCatcher, iCode Systems Ltd., UK, between 2011 and 2016) to trigger recording only when movement was detected. Video data was reviewed daily to assess activity at the burrow while manual observation of burrows without cameras was made. Cameras were removed from burrows that became vacant and redeployed at occupied burrows daily throughout the season. This ensured that, as much as resources allowed, all occupied burrows were monitored by video cameras.

Cricket Tagging and Morphological Measurements

Adult crickets were captured from their burrows from two days post-emergence to fit identification tags and collect morphological and molecular data. Trapping was initially conducted using funnel traps which were replaced in 2013 with bespoke traps designed by Luke Meadows (see crickettrapping.wordpress.com for details). Traps were left in burrows while the occupying cricket was captured to prevent the burrow being taken over by other individuals. Each individual was weighed using a precision balance (SNUG II-300, Jadever, Taiwan) and notes on physical condition were made such as missing, injured or deformed legs, antennae or cersi. A PVC tag bearing a 1-2 character code, unique to each

individual, was glued to the pronotum of each individual using cyanoacrylate, after scratching away the top layer of the pronotum cuticle with a craft knife thereby allowing individuals to be identified from video data. Top-down digital photographs were taken of the head and thorax of each individual using a digital camera (PL210, Samsung, South Korea) mounted on a dissecting microscope (S6E, Leica Microsystems, Germany). These photographs were produced without the use of anaesthesia by placing the cricket in a cavity cut in a wooden disk covered by a clear glass plate to prevent movement or escape. Three photographs were taken of each individual and the cricket was allowed to adjust its position slightly between each with the average measurement being used in analyses to avoid errors caused by the angle of the cricket. The width of the pronotum at its widest point, perpendicular to the anteroposterior plane, was measured using the image analysis software, FIJI (Schindelin et al., 2012). This distance was measured in pixels and calibrated by comparing this distance to the pixel distance of the character length on the identification tag, for which the exact distance was known in millimetres (see Figure 4). Pronotum width has been shown to be highly correlated with overall body size in crickets and so can be used as a reliable proximate measure of body size (Simmons, 1986b). All images per cricket were analysed and the average pronotum width for each individual was used as its measure of body size. Additional procedures conducted during capture, but not relevant to this thesis are: amputation of the final segment of the hind leg for DNA analysis, collection of haemolymph, and collection of cuticular hydrocarbon samples. Following a short period of recovery, crickets were returned to the burrow from which they had been captured.

Figure 4: Cricket morphology photograph as used in FIJI to measure pronotum width. The yellow line indicates the plane in which the pronotum was measured while the red line indicates the character measurement used to calibrate the pixel distance of the pronotum, and convert the measurement to millimetres.

Life history and behavioural monitoring

Behavioural observations were made throughout the reproductive season through manual playback of recorded video using iCatcher. The date, time and location of any of 19 events (ten behavioural and life history events, and nine system-related events, see Table 1 and Table 2) were manually extracted along with the IDs of any crickets involved to provide a detailed record of cricket behaviour across the adult lifespan of each individual.

Following each field season, video processing is conducted in three stages. The first stage is a filtering process by which sections of video containing no cricket activity are identified and ignored in subsequent stages. While the video cameras run continuously, the data is stored in discrete 24-hour periods, midnight to midnight. During stage one the observer reviews the data recorded from up to 20 cameras, from a single day, at high speed. If an adult cricket is observed on any camera, that camera on that day is marked to be watched in detail during stage two.

Behavioural and system data are collected during stage two. Up to four cameras are reviewed at once and the observer has control over the direction and speed of playback. All sections of video highlighted as containing cricket activity during stage one are reviewed, after which the collected data is subjected to error-checking in stage three.

In stage three, a series of queries are run in Microsoft Access (Microsoft Corporation, Washington, USA) to detect inconsistencies in the data collected. These may be occurrences of one individual being observed to arrive at a burrow twice consecutively without having been recorded to have left in between or occurrences of mating or fighting events without both individuals having been recorded as arriving, as examples. Stages two and three are reiterated until stage three no longer detects any inconsistencies. Only once this is achieved is the data subjected to analyses.

Table 1: List of behaviour and life history events recorded during manual playback of video data, along with the description of those events.

Behavioural and Life History Events	**Description**
Emergence	Cricket emerges as adult from its final nymphal instar.
Arrives	A cricket, not currently occupying the burrow, comes in to view of the camera.
Leaves	A cricket moves out of view of the camera for a continuous period of more than five minutes.
Fights	Two crickets of the same sex engage in an agonistic interaction. Agonistic interactions are considered to range from the exclusion of one individual with neither displaying aggressive behaviour to one or both individuals displaying aggressive behaviour. Aggressive behaviour includes all threatening behaviour from antennation to mandible flaring to biting and wrestling.
Mates	The female mounts the male in the mating position for at least several seconds, irrespective of spermatophore transfer. Spermatophore transfer is confirmed or refuted as an additional record.
Removes Spermatophore	A female deliberately removes a spermatophore from her ovipositor using her mandibles.
Oviposition	A female deposits eggs around the burrow by forcing her ovipositor in to the ground.
Attacked by…	A predator injures or attempts to eat a cricket, or penetrates an active burrow, but the cricket is not killed. Species of predator is noted.
Predated by…	A predator kills, eats, or removes a cricket from the view of the camera. Species of predator is noted.
Dead	A cricket is seen to be dead by some cause not known to be predation.

Table 2: List of system-related events recorded during manual playback of video data, along with the description of those events.

System Events	**Description**
On	The camera begins recording at the start of the season, or after a period of being off.
Off	The camera stops recording.
Present	A cricket is already occupying a burrow when the camera is turned on.
Out	A cricket is occupying a burrow when the camera is turned off.
Relocated	A camera is moved to a different burrow.
Trapping Starts	A trap is placed in a burrow.
Trap Checked	The trap is checked for cricket capture.
Trapping Ends	The trap is removed from the burrow and any captured crickets are returned.
Tagged	A cricket has been tagged during the preceding period of capture.

Quantification of male calling song

Calling effort for each male was quantified by reviewing the first 10 minutes of each hour of video data identified as featuring cricket activity during stage two. Male *G. campestris* call in relatively long bouts and so sampling at one-minute intervals throughout the 10-minute observation period was found to provided sufficient resolution to detect calling behaviour. Individuals could be seen calling from still images as the wings are raised to an approximately 45-degree angle to the body. This is an easily recognisable position and does not occur for any other behaviour. Male calling behaviour was recorded as a binary response indicating if the male was observed to call during the 10-minute observation period. The investment in calling made by each male across his adult life could then be quantified as the number of observation periods in which he was observed to call relative to the total number of observations made for that male.

Statistical Analyses

All statistical analyses were performed in R version 3.3.2 (R Core Team, 2016), primarily using general and generalised linear models (GLM), and generalised linear mixed models (GLMM). Exceptions include one hierarchical generalised linear model (HGLM) performed in Chapter Three, and one binomial test performed in Chapter Five. For each analysis, the distribution of the response variable was assessed visually as a histogram. When the data did not fit the normal distribution, the appropriate link function for the distribution was applied to the model. Maximal models were built and the fit of the model was assessed visually from plots of model residuals using the plot() function in R. Where appropriate, models were checked for over- and under-dispersion and variance inflation caused by potential collinearity (details are provided in the methodology

sections of the relevant chapters). Models were simplified, where required, through step-wise deletion of terms; first removing interaction terms and then the least significant term until the adequate minimal model was reached. The added deviance caused by the deletion of terms was checked using the anova() function as according to Crawley (2005). The removal of the term was upheld if there was a non-significant increase in model deviance. Reported p values were obtained by comparing models excluding and including the term of interest using the anova() function. GLMs were fitted using base R packages while GLMMs were fitted using the package “lme4” (Bates et al., 2015). All plots have been produced using the package “ggplot2” (Wickham, 2009).

Summary of Chapters

The novel work presented in this thesis address questions regarding the action of condition-dependent sexual selection in a natural environment. The following four chapters, summarised below, are presented as individual papers. They cover a direct experiment of the effect of condition on sexually selected traits and mating success, the influence of body size in determining mating success, the capacity of *G. campestris* to utilise alternative mate searching tactics in relation to condition, and the effect of female condition on mate choice.

Chapter Two: Condition dependence of male sexual traits and mating success: An experimental approach in a wild system

At present, it is not clear under which circumstances sexual selection will accelerate or retard adaptation. I am not aware of many studies showing evidence of sexual selection accelerating adaption in a natural environment, and laboratory experiments, performed under artificial conditions, may

underestimate the benefits of sexual selection in a changing environment where heritable variation for fitness is increased (Holland, 2002; Lorch et al. 2003). In Chapter 2 I report an experiment conducted on a wild population of *G. campestris*. Adapting the methods of a similar study by Holzer et al. (2003), I provide half of the population with a dietary supplement while late-stage nymphs (and continuing in to adulthood in a second field season). Dietary supplementation has been shown to be a useful method of manipulating individual condition in laboratory systems (Hunt et al., 2004, Judge et al., 2008) and so I investigate its effect in the wild. Through intensive observation I ask, does dietary supplementation affect (1) the development of *G. campestris*, (2) life history and sexually selected traits of males, and (3) male mating success.

Chapter Three: Body size fails to predict mating success in a population of wild field crickets

Body size is frequently reported to influence male attractiveness or be correlated with sexually selected traits (see highlighted studies in Table 4). Body size itself can be considered a condition-dependent trait for the same reasons that energetic displays or ornament signals are – to be a larger individual you are expected to have been able to utilise more resources and therefore are expected to have advantageous genes (Emlen and Oring, 1977;. Rowe and Houle, 1996). Many of the studies that address the role of body size in mating success are conducted under laboratory conditions, losing the effect of environmental variation or perhaps even natural variation of the animals themselves. In chapter 3 I investigate the role of body size in determining mating success by observing a population of *G. campestris* over nine

reproductive seasons with a monitoring intensity not previously available for a wild insect.

Chapter Four: Male mate-searching tactics are affected by context but not condition in a population of wild crickets

Condition-dependent signalling allows high-quality individuals to achieve greater fertilisation success than lower-quality rivals. Often, however, males have multiple routes by which fertilisation can be achieved which may be mediated by the condition of the individual. In some Gryllid species, males are known to have at least two tactics by which females can be encountered: signalling and searching (Cade and Cade, 1992). Signalling males remain at their burrows and call to attract female for mating, while searching males silently roam their surroundings and encounter females by chance. Here I investigate if males who are outcompeted acoustically, might switch to the searching strategy in order to increase their mating success by investigating a potential trade-off between calling behaviour and movement indicative of a condition-dependent, strategic switch-point. Following on from studies in other Gryllid species, I also investigate environmental effects on mate searching tactics in *G. campestris* by looking among reproductive seasons which vary naturally in population size, and observe changes in the amount of male calling associated with population density.

Chapter Five: Within- and among-individual variation in female mate choice in wild crickets

While studies of sexual selection often focus on variation in males, in many systems, mating success is largely driven by female choice. As such, to build an

accurate picture of the mechanisms of sexual selection it is important to consider the influence that females have – and the effect that female condition may have on male mating success, and therefore on sexual selection. In chapter five I investigate the effect of among- and within- individual variation in female mate choice in relation to individual condition and mating history. Through close observation of naturally occurring mating events I look at variation in female choosiness in relation to her mass, size or condition index, and well as variation in choosiness between virgin and mated females.

Chapter Six: General Discussion

In chapter six I present my general discussion of this thesis, drawing together the insights from each chapter and the implications of these for sexual selection theory. I further discuss the importance, and difficulties, of studies on wild systems and provide considerations for the future employment of such studies.

Chapter Two

Condition dependence of male sexual traits and mating success: An experimental approach in a wild system

Abstract

Natural and sexual selection are two major evolutionary forces which can lead to adaptation but are often considered to act in opposition; sexually selected traits are often costly in terms of survival, so natural selection is expected to act against them. If, however, natural and sexual selection act in concert, this is expected to accelerate adaptation to changing environmental conditions.

Individuals are expected to prefer high-quality mates to obtain the direct or indirect benefits associated with mating with such individuals. Sexually selected condition-dependent traits, where the magnitude of the trait is constrained by the condition or quality of the individual, offer a mechanism by which the choosing sex can determine the quality of potential mates. As such, traits that confer high-quality will spread due to their sexually selected preference advantage as well as their naturally selected quality advantage. This has the potential to align natural and sexual selection and therefore drive rapid adaptation.

I investigated the role of sexual selection in adaptation by exploring the effect of condition on sexual traits and mating success in a wild population of *G. campestris*. Cricket condition was manipulated by providing different levels of dietary supplementation and behaviour was monitored using a network of video cameras. I investigated the effect of dietary-supplementation on calling effort (a primary method of mate attraction in this species), mating success (as an indicator of reproductive success), and how soon after emergence males begin mating. I also investigate the effect of dietary supplementation on body mass, body size, lifespan, and fighting ability.

Food-supplemented, high-condition males did not begin mating sooner than control males, nor did they obtain more mates or achieve more mating events. Food-supplemented males invested more in calling effort but this increase was not sufficient to produce differential mating success in relation to food supplementation. My finding that dietary-supplementation increases calling effort supports the idea that this is a condition-dependent trait that could align natural and sexual selection. However, the fact that this does not lead to increased mating success for highly supplemented males suggests that either my dietary-supplementation treatment does not generate a biologically significant increase in calling effort, or that females have additional mechanisms by which to assess males.

Introduction

Rapid changes in prevailing environmental conditions can have drastic effects on the viability of natural populations. Changes in environmental conditions can cause previously beneficial alleles to become unfavourable and reduce population viability, or disrupt the cost-benefit structures of traits (Candolin and Heuschele, 2008). Populations experiencing significant environmental change must respond or else face extinction. Globally, animal populations are being seen to shift their geographical ranges to more extreme latitudes (Chen et al., 2011, Poloczanska et al., 2013), or temporally shift the timing of migration (Baker et al., 2015, Filippi-Codaccioni et al., 2010, Kovach et al., 2013) or reproduction (Both et al., 2004, Green, 2017) in order to keep within their preferred environmental limits. Such behavioural responses offer rapid solutions to environmental change; however, they are not available to all populations nor are they limitless (Both and Visser, 2001, Sinervo et al., 2010). Eventually behavioural change may not be sufficient, at which point the persistence of natural populations will depend on their ability to adapt to new conditions.

Natural and Sexual Selection in Adaptation

Natural and sexual selection are two major forces driving evolution which have a large effect on the frequency of alleles in a population. Natural selection favours phenotypes that provide the greatest advantage to an individual's survival and acts on nonsexual fitness components such as development, condition, and behaviour. Sexual selection is a similar yet distinct process that acts on the individual's capacity to secure investment in their offspring from mates (Andersson, 1994). Sexual selection does not necessarily act to increase survival, but drives individuals to be better able to secure fertilisations, for

instance by being more attractive (Andersson, 1994). Novel environmental conditions can have significant effects on the actions of both natural and sexual selection by changing the costs and benefits of trait expression. A classic example is the change in frequency of dark versus light colour morphs of Peppered Moths (*Biston betularia*) in the United Kingdom during the industrial revolution. Previously, the light morph was advantageous as it provided better camouflage against the bark of trees. As tree bark was darkened by soot following increased levels of air pollution from industrial activity, natural selection favoured the darker morph (Haldane, 1932). More recently it has been shown that increases in global temperature are changing the cost benefit structure of a sexually selected trait in African Lions (*Panthera leo)*. Males with darker manes typically have greater mating success, despite the cost of darker manes increasing body temperature. Rising temperatures however, are increasing the cost of darker manes such that the sexually selected benefits may be outweighed by the naturally selected costs (Patterson et al., 2006).

Where natural and sexual selection favour different optimal levels of trait expression, the result is misalignment of directional selection. For example, a brightly coloured bird may obtain more mates than his rivals due to his colouration. Brighter feathers would therefore be selected for by sexual selection. Bright colours are more conspicuous however, so such individuals may experience greater predation risk causing natural selection to select against them. When natural and sexual selection act in opposition, sexual selection is expected to promote maladaptive traits and slow the rate of adaptation. The rate at which a population can adapt to a novel environment

may therefore be affected by the interaction between natural and sexual selection.

Condition Dependence and Indicator Traits

The traditional view of the role of sexual selection in adaptation suggests that it is misaligned with natural selection (Darwin, 1871), however recent research has given support to an adaptive role of sexual selection (Fricke and Arnqvist, 2007, Lorch et al., 2003, Radwan et al., 2004, Veen and Otto, 2015). Due to the different reproductive costs for males and females that arise from anisogamy, it pays females to be selective in the mate that they choose (Parker et al., 1972, Trivers, 1972). Discriminately choosing superior males can provide a female, or her offspring, with a fitness advantage and so her definition of an attractive male should be adaptive (Hamilton and Zuk, 1982, Weber et al., 2007, Zuk et al., 1990). Indicator trait hypothesis models, also known as good genes (Trivers, 1972) or handicap hypotheses (Zahavi, 1975), suggest that female mate choice should be based on useable information about a potential mate, advertised by some signalling trait. A female may be able to assess the resources she can gain directly from a male - such as nuptial gifts (Cratsley and Lewis, 2003) or preferential territories (Keyser and Hill, 2000, Part and Qvarnstrom, 1997), the male's ability to contribute to raising her offspring (Buchanan and Catchpole, 2000, Hill, 1991, Knapp and Kovach, 1991) – or indirect, or genetic benefits such as identifying well adapted males whose genes she can capture for her offspring (Welch et al., 1998)

The stability of an indicator trait system is dependent on the reliability of the signal in honestly communicating male quality. If excessive cheating is present

within the population, females are as likely to mate with low quality males than high quality males, and so the benefit of preferring the display trait is lost (Grafen, 1990a, Johnstone and Grafen, 1993, Zahavi, 1975). When signalling traits are under directional selection they will eventually become condition dependent as further exaggeration will incorporate variation in the ability of individuals to acquire and allocate resources (Rowe and Houle, 1996). Condition-dependent signals ensure honesty as the level of signalling that an individual can maintain is constrained by its physical condition. High condition males, who have managed to acquire more resources than are required to survive, can invest more in sexually selected display traits. Lower condition males, unable to secure as many resources, cannot invest as much in displays without incurring intolerable costs and so are constrained to display at lower levels (Grafen, 1990a, Grafen, 1990b, Zahavi, 1975). The overall condition of an individual is determined by multiple genetic loci which affect the overall performance of the individual in the given environment (Houle, 1991, Price and Schluter, 1991, Rowe and Houle, 1996). Performance determines the ability of an individual to acquire resources which is a major component of condition (Rowe and Houle, 1996). Females can then use condition-dependent signals to choose high quality males and secure his genes for her offspring. When females choose males based on underlying condition, it follows that sexual selection should aid in adaptation to a new environment as beneficial alleles spread in the population due to their naturally selected benefits but also their sexually selected advantage; making sexual selection a potentially powerful adaptive force.

Mixed evidence for the adaptive role of sexual selection

While an adaptive role of sexual selection has been the focus of much research in recent years no clear consensus has been reached. The theoretical capacity for sexual selection to accelerate adaptation has been demonstrated (Lorch et al., 2003), however empirical studies spanning a broad range of taxa are mixed in their conclusions. Experimental studies in flies, beetles, amphibians, mites, crickets and fish have failed to reach a consensus, even when experiments are conducted on the same taxa (See Appendix 1 for details). Further studies on rodents and birds have found support for a positive role of sexual selection, while studies in yeast have found sexual selection to impede adaptation (Appendix 1).

There are both biological and methodological explanations for why studies may fail to detect an adaptive role of sexual selection. Biologically, one possibility is the effect of the study population's evolutionary history, and what mechanisms are maintaining genetic variation. Genetic variation can be maintained by the input of deleterious alleles caused by, for example, mutation, environmental change or immigration. Condition is expected to be sensitive to mutation as it is determined by a large portion of the genome, and so presents a large mutational target (Rowe and Houle, 1996). It follows therefore that mating success should be biased in favour of adaptive alleles. If so, selection is directional and natural and sexual selection are expected to operate in concert. Genetic diversity may alternatively be maintained by sexual antagonism, where beneficial alleles in one sex are deleterious in the other. In such a scenario it is expected that selection will act to balance allele frequency at some intermediate level with natural and sexual selection acting against one another (Long et al.,

2012). The contribution of each of these mechanisms to standing genetic variation can have an important effect on whether or not sexual selection is likely to perform an adaptive role (Long et al., 2012).

It follows also that the adaptive role of sexual selection may be underestimated in systems exhibiting high levels of sexual conflict. In polygamous species where males are in continual competition for fertilisations, male-induced harm and other sperm competition avoidance tactics are observed (see Danielsson, 1998). These traits are advantageous to the male as they increase his paternity share, but are associated with obvious disadvantages for the female. Female choice may therefore select less harmful traits more than indicators of high condition and so the purging of deleterious alleles by sexual selection is masked by the loss of fitness in females (Holland and Rice, 1998, Hollis et al., 2009).

Among the methodological explanations that adaptive sexual selection may not be detected is that many previous investigations have been conducted under laboratory conditions. While a vital tool in disentangling the complexity of biological phenomena, laboratory studies can be associated with important limitations. For example, laboratory stock populations are not expected to exhibit the same levels of genetic variance as a natural population might, dampening the effect of sexual selection that might otherwise occur in the wild (Holland, 2002). Furthermore, for most laboratory based studies, measurements on male fitness are often taken at a single point in time. Sample sizes are typically large enough to mitigate concerns about a male having an “off day”, however this methodology fails to consider interactions between male

performance and age, or the effects of repeated mating. Similarly, during artificial adaptation experiments, populations are often measured many generations apart rather than at each generational time step. As Hollis et al. (2009) points out, this approach is less likely to detect positive effects of sexual selection on adaptation as such effects are greater in early generations. After many generations, equilibrium in genotype frequency is more likely to have been reached and a greater proportion of genetic variance will come from sexual antagonism than from deleterious alleles (Fricke and Arnqvist, 2007, Long et al., 2012, Lorch et al., 2003). Additionally, artificial evolution experiments are often conducted using extreme, binary treatments – typically in the form of a benign versus stressful environment. While such an approach is a valid test of the general capacity of sexual selection to promote adaptation, the generality of the results depends on how realistic the treatments are.

A need for clarification

Forcing populations to adapt to experimental conditions that are profoundly different from their natural environment may cause extreme effects, increasing the likelihood of detecting adaptation. This approach may provide proof of concept but is not necessarily informative about the rate of adaptation under natural and gradual change. To investigate the ultimate contribution of sexual selection to adaptation in nature it is important to study wild populations.

In this chapter I attempt to resolve some of the uncertainties in studies caused by limitations of the laboratory environment. I do this by utilising a network of video cameras which allow me to intensively monitor a wild population of field crickets, *Gryllus campestris*, in their natural environment. In this system I employ a dietary supplementation experiment over several days in two

consecutive field seasons. Supplementary feeding has been shown to be an effective method of manipulating individual condition (Holzer *et al.*, 2003; Hunt *et al.*, 2004) and is expected to remove the selective pressure of resource acquisition for supplemented individuals, allowing each to acquire resources without any costs of foraging. This should increase the condition of supplemented individuals such that, if an indicator trait mechanism is operating in this system, these individuals will be able to allocate more resources to condition dependent traits. It is predicted that increased provisioning with a dietary supplement will therefore increase the size and mass of these individual and increased resource available should result in greater expression of sexually selected traits males associated with supplementation (Rowe and Houle, 1996). Food supplemented males are therefore expected to be phenotypically analogous to optimally adapted individuals and should therefore achieve greater mating success (measured here as the number of mates obtained and the number of times mated) than males not receiving the supplement. To address these predictions I investigate the extent to which food-supplementation increases: calling effort, mating success and victories during contests, as well as the effect food supplementation has on development.

Methodology

The WildCrickets Field Site and Behavioural Monitoring

The following experiment was conducted at the WildCrickets field site in Asturias, Spain during the 2015 and 2016 reproductive seasons. The study population of field crickets, *G. campestris*, which have been established here for over 40 years (Rolando Rodriguez-Muñoz *pers. comms*), occupy an 800 m^2 area of relatively flat grassland. Crickets create and take refuge in burrows dug into the substrate and it is expected that the vast majority of cricket behaviour takes place in the immediate vicinity of these burrows. Each year, these burrows are detected manually by researchers and identified by a unique number. One of up to 130 video cameras (VivoTek, Taiwan) are deployed at each burrow to record cricket behaviour. The video cameras are deployed prior to crickets emerging as adults and continue recording throughout the reproductive season until the last individual dies. The cameras are equipped with day-night capability and so run continuously throughout this time.

To enable identification of individual crickets, adults are trapped at their burrows, using bespoke traps (see crickettrapping.wordpress.com for details), from two days post-emergence and fitted with a PVC tag. The tag is glued to the pronotum using cyanoacrylate glue and bears a 1-2 character code unique to each individual, which is easily read during video playback. During trapping morphological measurements are also made; specifically individual mass using a precision balance (SNUG II-300, Jadever, Taiwan) and pronotum width. Pronotum width is used as a proximate measure of body size and is measured from top-down photographs of the pronotum made using a digital camera (PL210, Samsung, South Korea) connected to a dissecting microscope (S6E,

Leica Microsystems, Germany). Pronotum width is measured using FIJI image analysis software (Schindelin et al., 2012), and each measurement is calibrated to the character height of the ID tag (see Figure 4). Three photographs of each individual were made, with individual being allowed to slightly adjust its position between photographs, so that an average measurement per individual could be used. This accounts for among-individual error caused by each cricket's position relative to the camera. Following a brief period of recovery, crickets were returned to the burrow from which they were captured. Traps were left in burrows during capture to prevent the burrow being taken over by other individuals.

Data on cricket behaviour was extracted from the recorded videos by manual playback. Playback was conducted in a three-step process: first to detect video sections which contained cricket activity, secondly to extract behavioural data from the video, and finally to check and rectify inconsistencies in the behavioural record. During the second stage, any of 19 key events were recorded (See tables 1 and 2) along with the burrow these events occurred at, the date and time of the event, and the ID's of the crickets involved.

For a full account of the methods highlighted above please refer to Chapter One: General Methodology.

Condition Manipulation

Cricket condition was manipulated by providing 50% of the population with a dietary supplement. Food was delivered from bespoke feeders which protected a food pellet (NovoTab, JBL, Germany) from the ground and surrounding grass to prevent excess water absorption by the pellet. The feeders were made from a round, open-topped, plastic cap 25 mm in diameter with a gap of 18 mm on one side to provide access for the cricket (See Figure 5). Keeping the feeder open at the top was necessary as crickets, especially nymphs, prefer to eat in sunlight early in the day (*pers. obs.*). The feeder was held in place by a steel nail 45 mm long which was drilled through the base of the plastic cap. The remaining 50% of the population formed the control group. This group was provided with the same food supplement from an identical cap that had been wrapped in wire mesh (see Figure 5). This controlled for the presence of food at the burrow site while preventing the cricket from accessing the pellet inside.

It has been recognised that sophisticated nutritional geometry approaches are important in determining an optimal diet (Archer *et al.*, 2009) and so this would have been the most robust approach to adopt here, as has been done in other cricket species (Rapkin *et al.*, 2016). Providing a more complex food source however, further complicates the practicalities of food provisioning in the wild and so a simpler supplement, in the form of a balanced, commercially available food pellet was chosen. The food pellet (Novotab, JBL, Germany) is a commercial fish food and was chosen due to its compositional similarity to the food supplement used by a similar study (Holzer *et al.* 2003) and so allows the best comparison between this study and my own. The pellet form was chosen as opposed to the flaked form used by Holzer *et al.* 2003 as it was expected this

would be more resistant to environmental conditions in the meadow, such as wind and rain. The pellets used contained 43% crude protein, 8% crude fat, 1.9% crude fibre and 8.1% ash. Crude components were derived primarily from molluscs, crustaceans, vegetables and fish, with small amounts of yeast, egg and algae were also present.

Each day of the experiment, the feeders were placed outside the entrance of burrows known to contain crickets, prior to the crickets becoming active or as weather conditions became conducive to the experiment (as high winds and rain suppress cricket activity rendering food provisioning redundant). Every burrow was provisioned with its own feeder throughout the experiment, wherever possible. Feeders were washed with water before being moved to a new burrow when necessary. Once each feeder had been placed at its burrow, one food pellet was added to each of the treatment feeders. At the end of each day, the treatment food was removed and stored individually in 24-well plates. All feeders were then removed from the meadow. Food pellets remained in control feeders continually as these were enclosed and there was no risk of losing the food. The same food pellet was used repeatedly in each feeder until half of it had been consumed, or weather conditions had degraded it, after which it was replaced with a fresh pellet.

Figure 5: Image of the cricket feeders used to provide dietary supplementation throughout the experiment. The experimental feeder is on the left of the image while the control feeder, wrapped in mesh to prevent access to food, is shown on the right.

The experiment was run during the reproductive seasons of 2015 and 2016. During the 2015 season, food supplementation was provided from 6th April 2015 until the first adult emergence on the 28th April 2017. Feeders were placed at active burrows for between 2 hr 21 m and 8 hr 33 m per day between 8:30 and 19:30 GMT. Nymphs rarely move among burrows therefore designating burrows as fed or control was reliable in ensuring a consistent feeding regime for each nymph. Feeding was carried out daily except when adverse weather, such as heavy rain and high winds, prevented food provisioning. During the 2015 experimental period this occurred for 2 non-consecutive days. Each day, food pellets were dried at 45°C for 30 minutes and weighed using a precision balance (SNUG II-300, Jadever, Taiwan) to determine daily food intake for each cricket. This was found to be an ineffective measure of consumption as video

data revealed other invertebrates consuming the food pellet and was therefore abandoned ahead of the 2016 season.

Dietary supplementation was provided to both nymphs and adults during the 2016 field season. While crickets were nymphs, the feeding protocol remained the same as in 2015. Adult crickets begin to move among burrows within a few days post-emergence. These movements were monitored using video playback and physical observation, and the feeders provided at each burrow were adjusted accordingly, on a daily basis. This ensured that, as far as possible, crickets received a consistent food treatment. Feeders were placed at active burrows from 1st April 2016 until 18th May 2016. Feeding was conducted for between 5 hr 54 m and 8 hr 42 m per day between 09:30 and 17:30 GMT. Feeding was prevented by bad weather on 10 days throughout the experiment, with the longest consecutive disruption being three days. Individuals that were fed as nymphs were also fed as adults, and control nymphs became control adults. Some exceptions occurred where control adults moved to a burrow provisioned with the food supplement. In each instance, such individuals were recorded as being fed on that day.

It is possible that the allocation of resources will differ between the nymph and adult stage as the priority for nymphs is likely to be more towards growth and development, whereas in adults the priority may be weighted towards reproduction. The life stage at which the dietary supplement is administered may therefore interact with the effect the supplement has on sexually selected traits, narrowing the frame of this study. While there are studies which find the dietary conditions of juveniles to affect reproduction in adults (Boggs &

Freeman, 2005; Kleinteich *et al*., 2015; Plesnar-Bielak *et al.*, 2017), it has been shown that reproductive investment is independent of juvenile conditions in orthopterans (Branson, 2004; Zajitschek *et al*., 2009). The most informative approach here would then be to comprehensively investigate the effect of supplementary feeding performed across the individuals entire lifespan of the individual. This is not practical in this study however as young nymphs cannot be detected and adult movement among burrows becomes unmanageable as the season progresses. This limits the period where supplementary feeding can be performed to the weeks surrounding the period of adult emergence. While this narrows the scope of this study it is not expected that the life stage at which feeding is administered will have a prominent effect on the results of this study as the major sexually selected trait investigated is calling effort which is more likely to be sensitive to current energy reserves, reflecting recent dietary conditions, than the development of structures, which would be more sensitive to dietary history.

Quantification of male calling song

Male calling effort was estimated through additional video review. The first 10 minutes of each hour that a male cricket was observed by a camera was observed in 1-minute intervals. A binary response was recorded for calling behaviour if the male was observed to call at any time during this 10 minute observation period. This allowed calling effort to be estimated as a proportion of the number of observation periods a male was observed to be singing relative to the number of observation periods for that male. Calling behaviour could be determined from the video recordings due to the raised position of the wings which is distinct to this behaviour and does not coincide with any other

activities. A full account of this methodology is given in Chapter One: General Methodology.

Effect of dietary supplementation on development

Data on mass at emergence, pronotum width (as a proximate measure for body size) and emergence date were pooled across the 2015 and 2016 reproductive seasons to determine if dietary supplementation of nymphs produces basic differences between supplemented and control individuals. The data were normally distributed and were analysed using three general linear models with the number of days provided with the dietary supplement before emergence included as the independent variable in each case. These analyses were conducted on all individuals in each reproductive season, therefore sex and year were included as covariates to account for any sex-specific effects of supplementation, or variation between years.

Effect of dietary supplementation on male life history

As the effect of dietary supplementation on male condition is of greatest interest in this chapter, only data collected from males were used in further analyses. Furthermore, due to differences in feeding procedure between the two years, with only nymphs being fed in 2015 and feeding continuing in to adulthood in 2016, the data from each year were analysed separately.

It was hypothesised that males provided with the dietary supplement would begin investment in exploratory and reproductive behaviour sooner after emergence than those who did not receive the supplement. For data from 2016, the time intervals between emergence and leaving the burrow area, and

emergence and first mating, respectively, were recorded in days and modelled as a function of the number of days the male received the dietary supplement (pre- and post-emergence feeding being included separately in the model). The data were analysed using a generalised linear model with a Poisson error distribution. The time intervals between emergence and leaving the burrow, and first mating, for individuals in the 2015 season, were also analysed using generalised linear models. In this case, only the number of days fed pre-emergence were included as no post-emergence feeding was carried out. A Poisson error structure was used to model the number of days until the male's first mating, however a negative binomial error structure was used to model the number of days until the male left the burrow, as the latter violated the assumptions of the Poisson distribution (mean and variance for the number of days until the male left the burrow were uneven, resulting in overdispersion of the Poisson model).

The effect of dietary supplementation on sexually selected traits

The effect of dietary supplementation on two potentially sexually selected traits, calling effort and fighting ability, were investigated. Calling effort was quantified as described in **Chapter One** and included as the proportion of times the male was observed singing, relative to the total number of observations made. For data from 2016, the number of days the supplement was received pre-emergence and post-emergence, respectively, were included as covariates. For data from 2015, only the number of days pre-emergence could be included. The data were modelled using generalised linear mixed models, each using a binomial error structure. Individual ID was fitted as a random factor in both models to control for repeated measures of the same individuals.

To determine the effect of dietary supplementation on the probability of winning a fight, the probability that a given individual would win was modelled as a function of the difference in the number of days supplemented relative to his opponent. Using the difference between two individuals is superior to using the absolute number of days the focal individual received the dietary supplement as it incorporates information on the condition of the opponent. For data from 2016, the difference in days supplement pre- and post-emergence were included as separate predictor variables. Individual ID was not included in the model for either year as the independent unit of measurement is the fight between two individuals, rather than either individual alone. To avoid pseudoreplication arising from repeated observations of the same pair, only the first fight between pairs, regardless of the role that either cricket played, was used in the analysis. Individual crickets may be part of several pairs, but never with the same opponent. The resulting dataset included 42 fights in 2015 and 38 fights in 2016 across 34 individual males in either year.

In cases when the number of days fed was not available for an attacking cricket, the mean number of days was used instead. Making comparisons against the mean is analogous to using the absolute number of days fed per individual, which would allow prediction of the average outcome when an individual supplemented for a given number of days competes against any given individual. Making comparisons against the true number of days that an opponent received the supplement, provides the model with additional information. In both years the data were analysed using a generalised linear model with a binomial error function.

Effect of dietary supplementation on male mating success

Male mating success was measured both as the number of unique mates that a male obtained, as well as the total number of times he mated throughout his life, irrespective of repeated mating events with the same female. The number of mates that a male obtained was modelled as a function of the number of days he was provided with the dietary supplement (as a nymph and as an adult for crickets in 2016, and as a nymph for crickets in 2015). As the data did not meet the assumptions of the Poisson distribution, a GLM was fitted with a negative binomial error structure. Similar models were produced for both years, respectively, to analyse the relationship between dietary supplementation and the total number of mating events achieved. These models were run using a Poisson error structure.

Results

Effect of Dietary supplementation on Development

The average mass of an adult cricket at the time of emergence was 0.98 g ± 0.012 (mean ± standard error). There was no difference in mass at emergence between males and females (Linear model: n = 96, $F_{1, 95} = 0.0058$, P = 0.94). Adult crickets of either sex were found to be approximately 0.003 g heavier at emergence per day supplemented as a nymph (Linear model: n = 96, $F_{1, 94} = 5.84$, P = 0.018, Table 1, Figure 6). Thus, the average mass of a cricket fed for 16 days was predicted to be 1.013 g ± 0.027, while the average mass of crickets who went unfed was predicted to be 0.96 ± 0.016. However, the number of days fed as a nymph had no significant effect on the body size of the adult cricket, measured as pronotum width (Linear model: n = 96, $F_{1, 92} = 0.14$, P = 0.71, Table 2). Sex and year had a small effect on adult size, with males being on average 0.37 mm larger than females (Linear model: n = 96, $F_{2, 92} = 8.64$, P < 0.001, Figure 7) and 2015 adults being on average 0.62 mm larger than 2016 adults (Linear model: n = 96, $F_{2, 92} = 24.66$, P < 0.001, Figure 7). While there was no significant effect of dietary supplementation alone on the timing of emergence, a significant interaction with year (linear model: $F_{1, 88} = 4.44$, P = 0.038, Table 3, Figure 8) suggests that there is some undetected difference between the years.

Table 3: Table summarising the analysis of the effect of supplementary feeding of nymphs, sex, and year on body mass at emergence. The table gives the formula of the maximal model and of the minimal adequate model obtained following stepwise simplification as well as the sample size and the specified error structure of the model. The table of coefficients from the minimum adequate model is given providing the estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest.

Maximal model	Body mass at emergence ~ Number of days fed as a nymph * Sex * Year			
Minimum adequate model	Body mass at emergence ~ Number of days fed as a nymph			
Sample size	96			
Model error structure	Gaussian			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	F	P
Intercept	0.96	± 0.0299		
Number of days fed as a nymph	0.003	± 0.0024	5.843	0.018

Table 4: Table summarising the analysis of the effect of supplementary feeding of nymphs, sex, and year on body size. The table gives the formula of the maximal model and of the minimal adequate model obtained following stepwise simplification as well as the sample size and the specified error structure of the model. The table of coefficients from the minimum adequate model is given providing the estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest.

Maximal model	Body size ~ Number of days fed as a nymph * Sex * Year			
Minimum adequate model	Body size ~ Sex + Year			
Sample size	96			
Model error structure	Gaussian			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	F	P
Intercept	7.76	0.1516		
Year (2016)	-0.62	0.1724	49.632	< 0.001
Sex (Male)	0.37	0.1742	17.308	< 0.001

Table 5: Table summarising the analysis of the effect of supplementary feeding of nymphs, sex, and year on date of emergence. The table gives the formula of the maximal model and of the minimal adequate model obtained following stepwise simplification as well as the sample size and the specified error structure of the model. The table of coefficients from the minimum adequate model is given providing the estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest.

Maximal model	Date of emergence ~ Number of days fed as a nymph * Sex * Year			
Minimum adequate model	Date of emergence ~ Number of days fed as a nymph * Year (2016)			
Sample size	92			
Model error structure	Gaussian			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	F	P
Intercept	125.86	1.3067		
Number of days fed as a nymph	-0.08	0.1158		
Year (2016)	-1.12	1.9347		
Number of days fed as a nymph:Year	0.16	0.1526	4.445	0.038

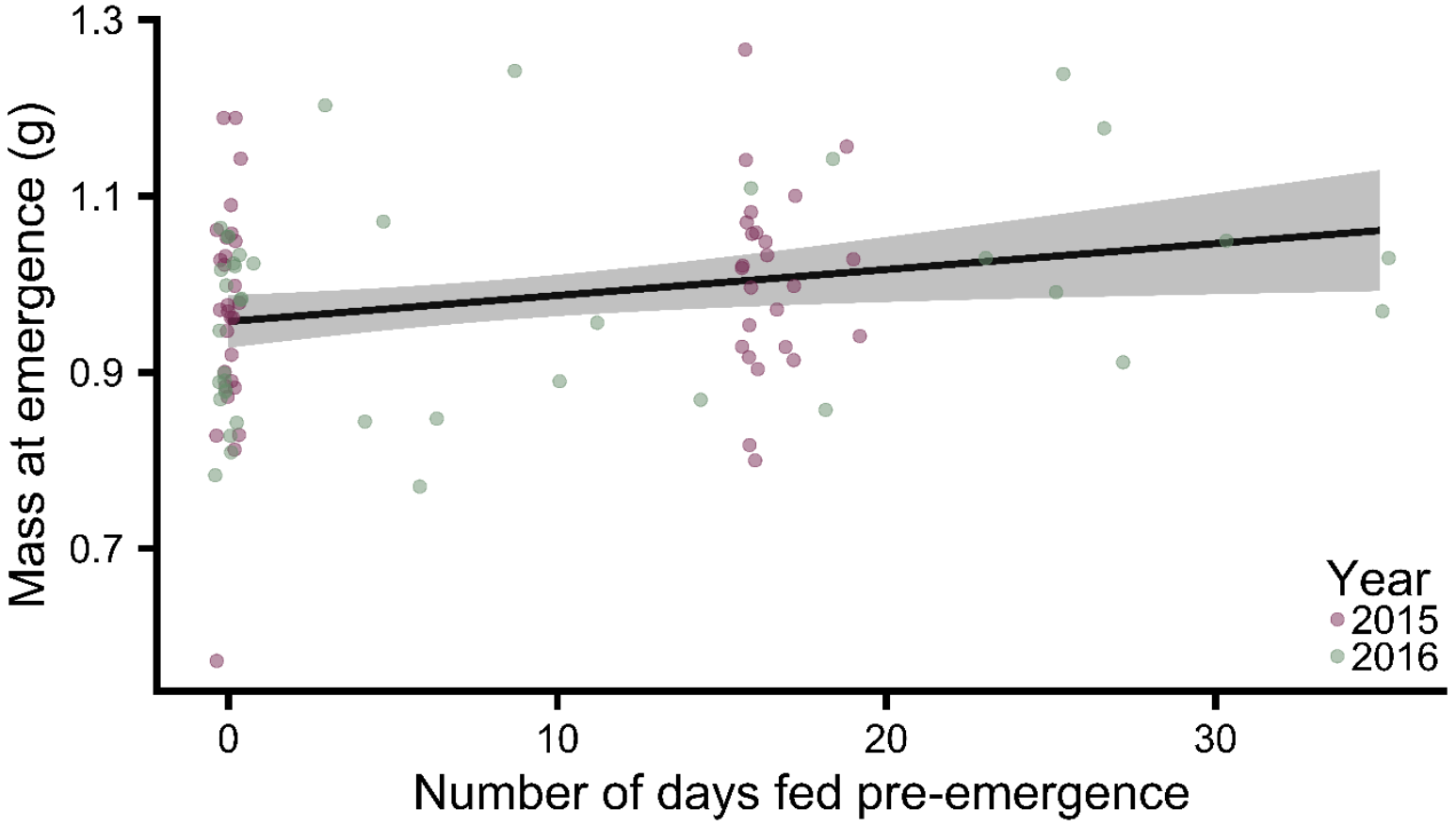

Figure 6: Plot showing the predicted increase in mass at emergence associated with the number of days an individual receives the dietary supplement as a nymph. The slope and intercept of the prediction line was calculated by linear regression of the number of days fed as a nymph on mass at emergence. The shaded area represents the 95% confidence intervals of the model and points represent the raw data collected in 2015 and 2016.

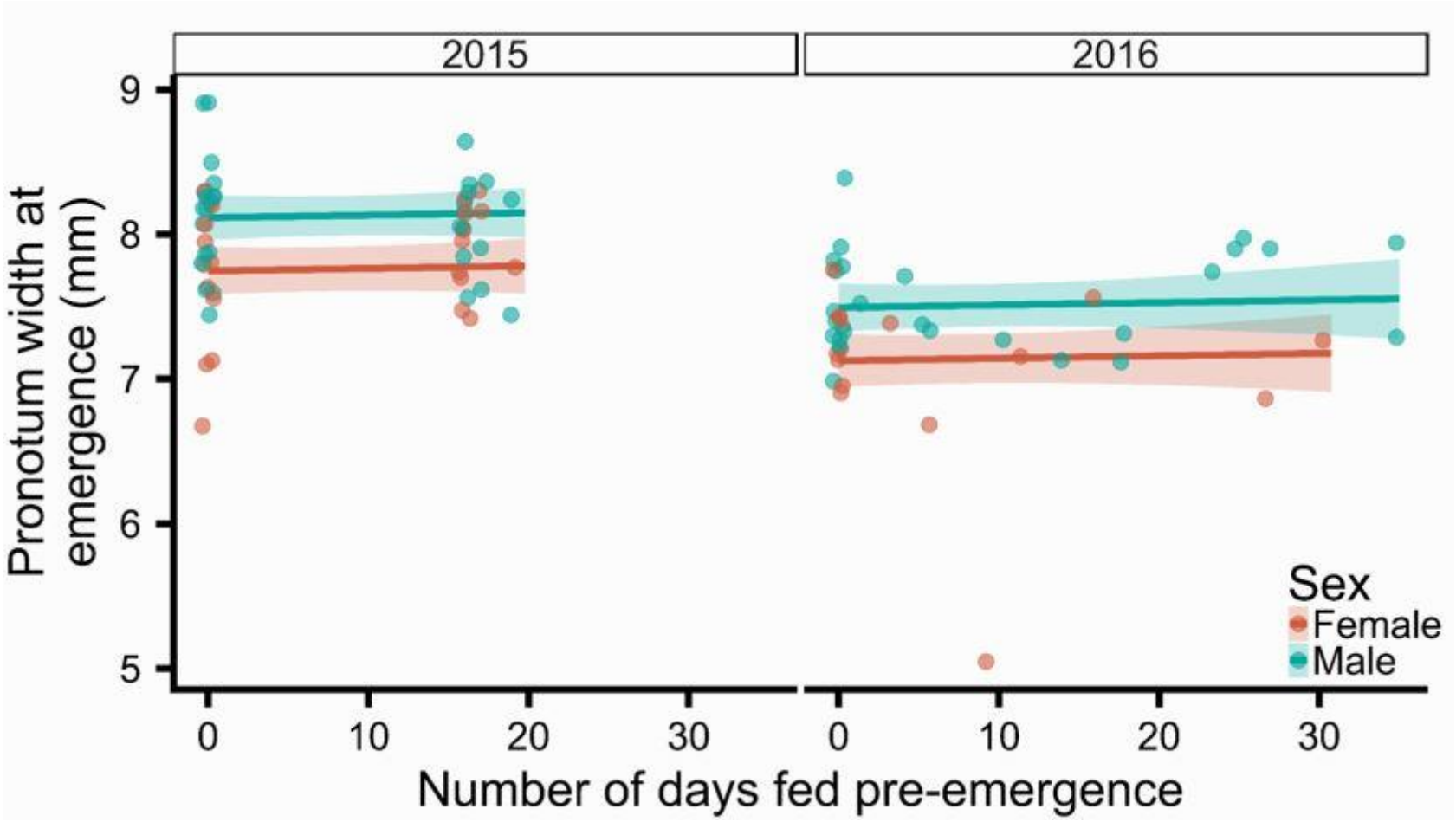

Figure 7: Plot showing the non-significant effect on adult pronotum width of the number of days nymphs were provided with the dietary supplement. Linear regression of Number of Days Supplemented, Sex, and Year on pronotum width revealed significant effects of Year and Sex. Shaded areas represent the 95% confidence intervals of the model and points represent the raw data.

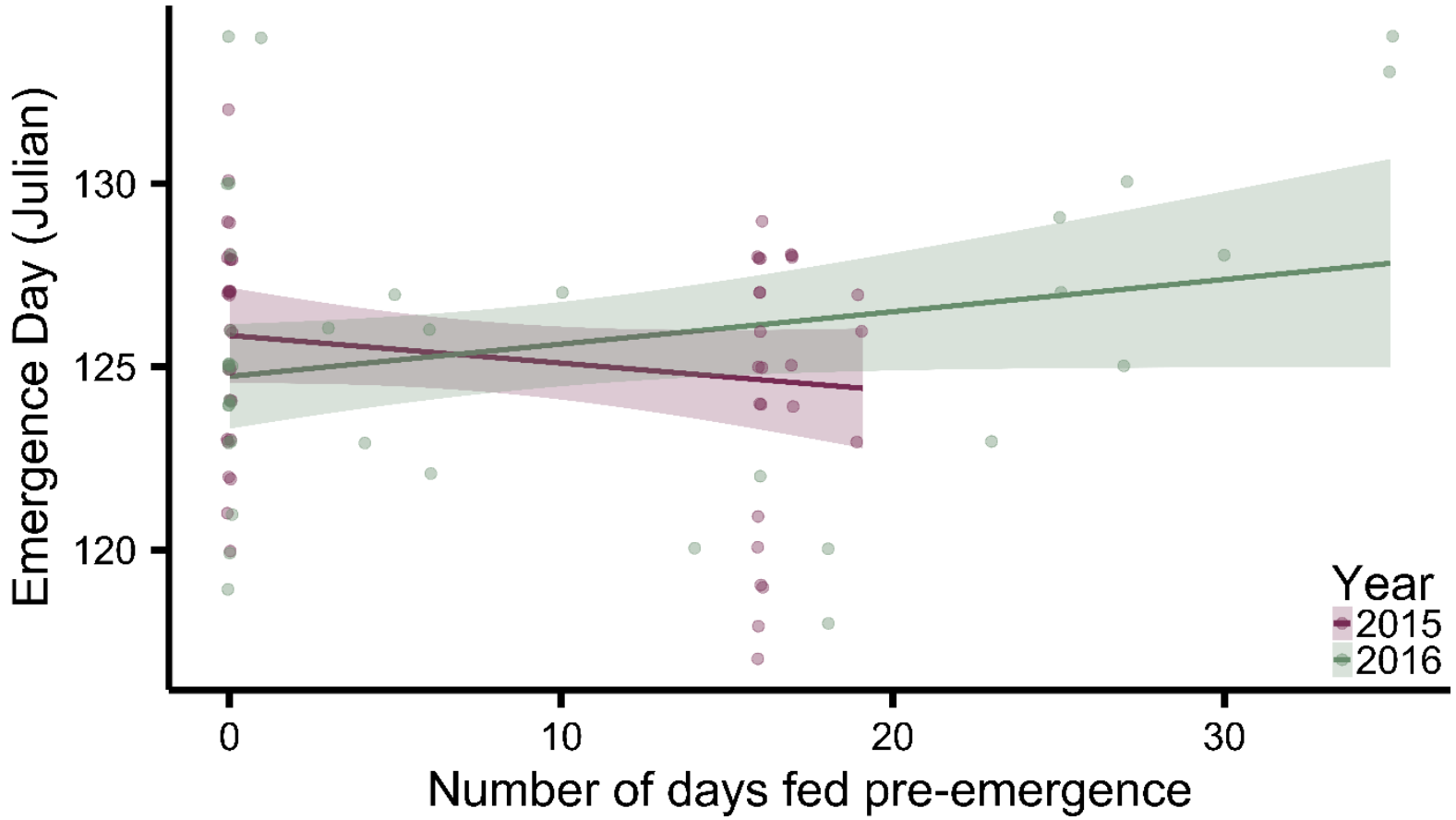

Figure 8: Plot showing the predicted change in Julian day of emergence with increasing number of days the dietary supplement was provided to nymphs in 2015 and 2016. Slope and intercept of the lines are calculated from a linear regression of an interaction term between Days supplemented and Year on day of emergence. Shaded areas represent the 95% confidence intervals and points represent the raw data.

Effect of Dietary Supplementation on Male Life History

The number of days until males left the burrow at which they emerged ranged between 0 and 16 days in 2015 and between 2 and 12 days in 2016. Per day supplemented as an adult, the number of days a male remained at this burrow increased at a rate of $e^{0.1076}$ (GLM: n = 21, χ^2 = 17.31, d.f. = 1, P < 0.001, Table 6, Figure 9). There was no significant effect of the number of days supplemented as a nymph in 2016 (GLM: n = 21, χ^2 = 0.2, d.f. = 1, P = 0.66, Table 6) or in 2015 (GLM: n = 29, χ^2 = 0.06, d.f. = 1, P = 0.81, Table 6).

The number of days post-emergence until a males first mating ranged from 5 – 30 in 2015 and from 9 – 24 in 2016. The number of days fed as an adult had no significant effect on how soon a male mated after emergence (GLM: n = 16, χ^2 = 1.1, d.f. = 1, P = 0.3, Table 7) nor as a nymph (2016: n = 16, GLM: χ^2 = 0.62, d.f. = 1, P = 0.43; 2015: GLM: n = 22, χ^2 = 0.058, d.f. = 1, P = 0.81, Table 7). The distribution of lifespan in 2015 and 2016 was consistent with the negative binomial distribution and was analysed accordingly, finding that lifespan was not significantly affected by the dietary supplementation of adults (GLM: χ^2 = 0.13, d.f. = 1, P = 0.72, Table 8) or of nymphs (2016: GLM: χ^2 = 0.19, d.f. = 1, P = 0.66; 2015: GLM: χ^2 = 0.049, d.f. = 1, P = 0.82, Table 8).

Table 6: Table summarising the analysis of supplementary feeding of nymphs (in 2015 and 2016) and adults (in 2016 only), and monitoring effort on the age at which males first leave their burrow area. The table gives the formula of the maximal and minimum adequate models along with the sample size and the error structure. The table of coefficients from the minimum adequate model for 2016, and from the maximal model for 2015 (as no significant effects were detected) are given. The model estimate and 95% confidence interval, test statistic, and the P value for the term of interest are provided.

Data from 2016				
Maximal model	Age at first leaving event ~ No. days fed (adult) + No. days fed (nymph) + Monitoring effort			
Min. adequate model	Age at first leaving event ~ No. days fed (nymph)			
Sample size	21			
Error structure	Poisson			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	X^2	P
Intercept	1.31	0.2824		
No. days fed (nymph)	0.11	0.0472	19.101	< 0.001
Data from 2015				
Maximal model	Age at first leaving event ~ No. days fed (nymph)+ Monitoring effort			
Min. adequate model	Age at first leaving event ~ 1			
Sample size	29			
Error structure	Negative binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	1.89	0.5929		
No. days fed (nymph)	-0.007	0.0224	0.364	0.546
Monitoring effort	0.015	0.0225	1.628	0.202

Table 7: Table summarising the analysis of the effect of supplementary feeding of nymphs (in 2015 and 2016) and adults (in 2016 only), and monitoring effort on the age at which a male first mated. The table gives the formula of the maximal and minimum adequate models obtained following model simplification. The sample size and the specified error structure of the model are given. The tables of coefficients from the maximal models are given as no significant effects were detected in either reproductive season and provide the estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest.

Data from 2016				
Maximal model	Age at first mating event ~ No. days fed (adult) + No. days fed (nymph)+ Monitoring effort			
Min. adequate model	Age at first mating event ~ 1			
Sample size	16			
Model error structure	Poisson			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	2.44	0.3816		
No. days fed (adult)	0.024	0.0449	1.097	0.29
No. days fed (nymph)	-0.005	0.0134	0.617	0.43
Monitoring effort	0.0057	0.0145	0.582	0.45
Data from 2015				
Maximal model	Age at first mating event ~ No. days fed (nymph)+ Monitoring effort			
Min. adequate model	Age at first mating event ~ 1			
Sample size	22			
Model error structure	Poisson			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	2.74	0.5066		
No. days fed (nymph)	-0.0017	0.0136	0.058	0.81
Monitoring effort	-0.0029	0.0175	0.105	0.75

Table 8: Table summarising the analysis of the effect of supplementary feeding of nymphs (in 2015 and 2016) and adults (in 2016 only) on lifespan. The table gives the formula of the maximal and minimum adequate models obtained following model simplification. The sample size and the specified error structure of the model are given. The tables of coefficients from the maximal models are given as no significant effects were detected in either reproductive season and provide the estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest.

Data from 2016				
Maximal model	Lifespan ~ No. days fed (adult) * No. days fed (nymph)			
Minimum adequate model	Lifespan ~ 1			
Sample size	25			
Model error structure	Negative binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	3.34	0.4139		
No. days fed (adult)	-0.039	0.1336	0.125	0.663
No. days fed (nymph)	-0.0014	0.0408	0.190	0.663
Data from 2015				
Maximal model	Lifespan ~ Number of days fed as a nymph			
Minimum adequate model	Lifespan ~ 1			
Sample size	30			
Model error structure	Negative binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	3.29	0.2357		
No. days fed (nymph)	0.0023	0.0207	0.049	0.824

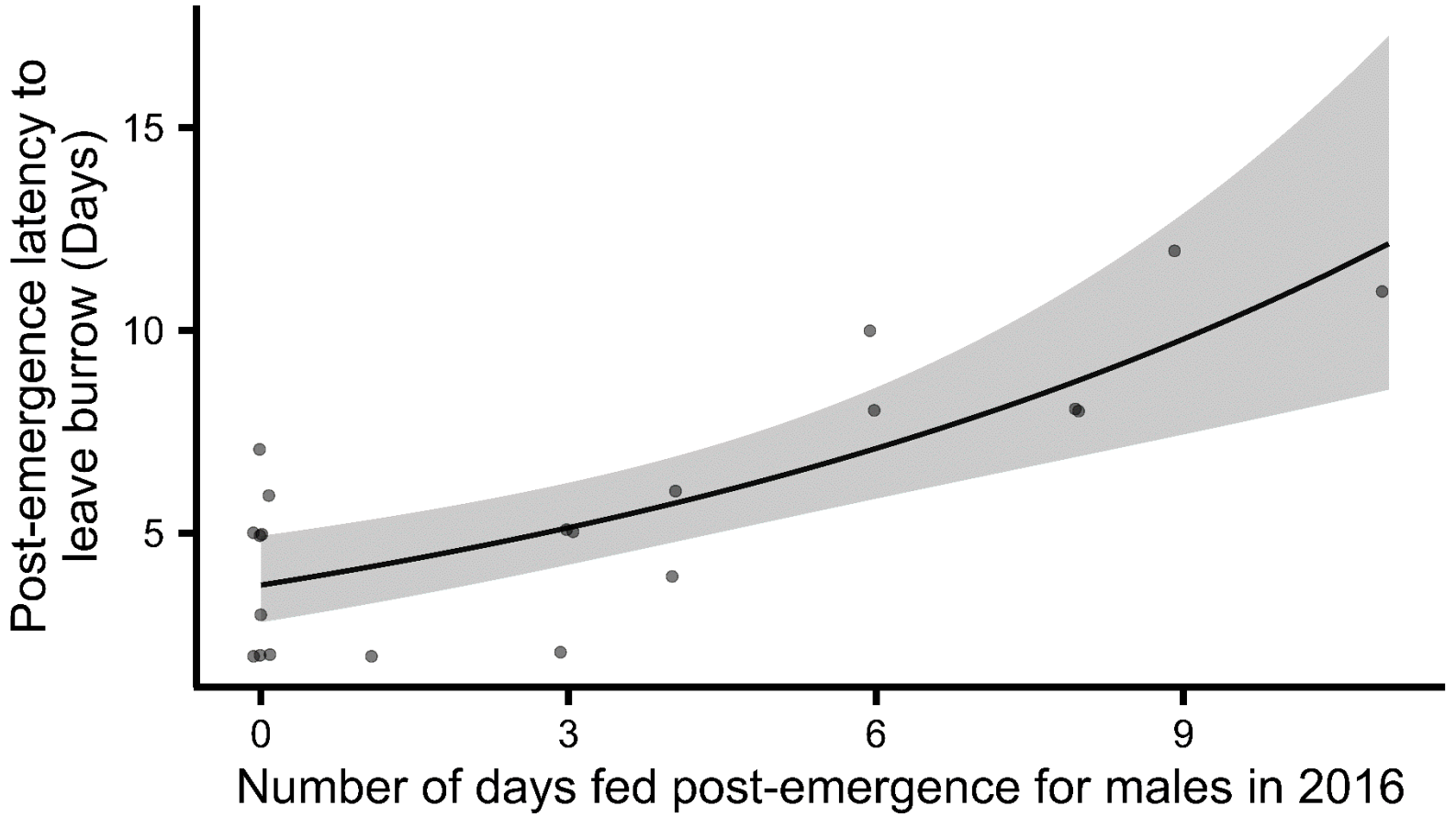

Figure 9: Plot showing the predicted increase in the number of days between emergence and leaving the burrow area for the first time in relation to the number of days provided with the dietary supplement as an adult for males in 2016. The prediction line is calculated from the minimum adequate model of a Poisson regression of the number of days fed as an adult on the latency to leave the burrow. The shaded area represents the 95% confidence intervals and points represent the raw data.

Effect of Dietary Supplementation on Sexually Selected Traits

Dietary supplementation of nymphs had no significant effect on calling effort in 2016 (GLM: $\chi^2 = 1.99$, d.f. = 1, P = 0.16) or 2015 (GLM: $\chi^2 = 0.14$, d.f. = 1, P = 0.71, Table 9). Adult males provided with the dietary supplement had a higher probability of singing than did control males (GLM: $\chi^2 = 468.63$, d.f. = 1, P < 0.001, Table 9, Figure 10). This effect was small, however, explaining just 27.9% of variation in calling effort. This is relative to the 48.2% of variation in calling effort that is explained by individual effects (obtained using the function rsquaredGLMM() from the R package MuMIn (Barton, 2016)).

Males who received the dietary supplement, as an adult or a nymph, for more days than their opponent during the 2016 season, were not more likely to win the fight (Days fed as adult: GLM: $\chi^2 = 2.45$, d.f. = 1, P = 0.12, Days fed as nymph: GLM: $\chi^2 = 1.07$, d.f. = 1, P = 0.3, Table 10). In 2015, being fed for more days as a nymph than an opponent produced a marginally non-significant increase in the probability of winning the fight (GLM: $\chi^2 = 3.74$, d.f. = 1, P = 0.053, Table 10).

Table 9: Table summarising the analysis of the effect of supplementary feeding of nymphs (in 2015 and 2016) and adults (in 2016 only) on calling effort, with male ID fitted as a random factor. The table gives the formula of the maximal and minimum adequate models obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the minimum adequate model is given for 2016, while the coefficients from the maximal model are given for 2015 as no significant effects were detected. The estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest are provided.

Data from 2016				
Maximal model	Proportion of time spent calling ~ No. days fed (adult) + No. days fed (nymph)+ (1|ID)			
Minimum adequate model	Proportion of time spent calling ~ No. days fed (adult) + (1|ID)			
Sample size	8838 observations across 25 individuals			
Model error structure	Binomial			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	X^2	P
Intercept	-4.41	1.091		
No. days fed (adult)	0.61	0.0727	468.63	< 0.001
Data from 2015				
Maximal model	Proportion of time spent calling ~ No. days fed (nymph) + (1|ID)			
Minimum adequate model	Proportion of time spent calling ~ 1 + (1|ID)			
Sample size	16,487 observations across 31 individuals			
Model error structure	Binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	-2.41	0.548		
No. days fed (nymph)	0.0093	0.0481	0.143	0.705

Table 10: Table summarising the analysis of the effect of supplementary feeding of nymphs (in 2015 and 2016) and adults (in 2016 only) on the probability of winning a fight. The table gives the formula of the maximal and minimum adequate models obtained following model simplification. The sample size and the specified error structure of the model are given. The tables of coefficients from the maximal models are given as no significant effects were detected. The estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest are provided.

Data from 2016				
Maximal model	Fight outcome ~ Difference in No. days fed (adult) + Difference in No. days fed (nymph)			
Minimum adequate model	Fight outcome ~ 1			
Sample size	38			
Model error structure	Binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	-2.18	0.6176		
Difference in No. days fed (adults)	-0.25	0.1733	2.449	0.118
Difference in No. days fed (nymph)	0.14	0.1743	3.483	0.062
Data from 2015				
Maximal model	Fight outcome ~ Difference in No. days fed (nymph)			
Minimum adequate model	Fight outcome ~ 1			
Sample size	42			
Model error structure	Binomial			
Table of Coefficients from the maximal model				
	Estimate	95% CI	X^2	P
Intercept	-1.088	0.7394		
Difference in No. days fed (nymph)	0.06	0.0638	3.743	0.053

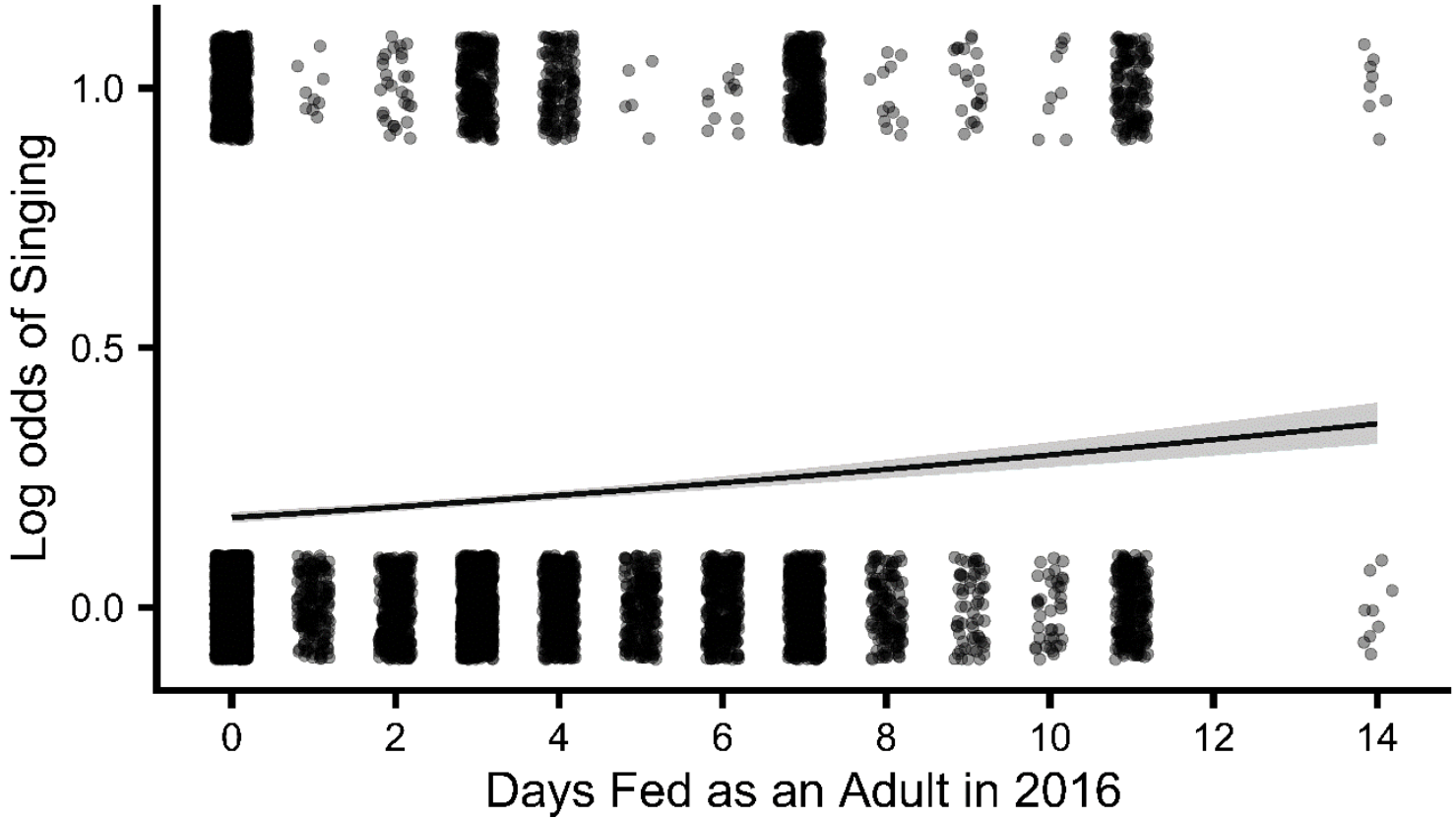

Figure 10: Plot showing the increase in the log-odds of an individual singing when observed in relation to the number of days fed as an adult for male crickets alive in 2016. The prediction line is calculated from the adequate minimal model of a logistic mixed effects model which included individual as a random effect. The shaded area represents the 95% confidence intervals of that model. Each point represents an individual sample and multiple samples are associated with each individual cricket. The outcome of each sample is either 1 or 0, but jitter has been introduced to show the density of samples.

Effect of Dietary supplementation on Male Mating Success

The median number of mates that males obtained was two, and the median number of mating events per male was four, for both seasons. Dietary supplementation had no significant effect on the number of mates that a male obtained in 2016, either by the number of days fed as a nymph (GLM: n = 26, χ^2 = 0.069, d.f. = 1, P = 0.93, Table 11) or the number of days fed as an adult (GLM: n = 26, χ^2 = 0.62, d.f. = 1, P = 0.43, Table 11). Across all mates that an individual male obtained, the total number of times that he mated throughout his lifetime was not significantly affected by dietary supplementation as a nymph (GLM: n = 26, χ^2 = 0.59, d.f. = 1, P = 0.44, Table 12) or an adult (GLM: n = 26, χ^2 = 2.94, d.f. = 1, P = 0.86, Table 12). Similarly, in 2015 the number of days fed as a nymph had no effect on the number of mates a male obtained (GLM: n = 31, χ^2 = 0.15, d.f. = 1, P = 0.7, Table 12), nor the total number of times that he mated (GLM: n = 31, χ^2 = 1.85, d.f. = 1, P = 0.17, Table 12).

Table 11: Table summarising the analysis of the effect of supplementary feeding of nymphs (in 2015 and 2016) and adults (in 2016 only), and monitoring effort on the number of mates a male obtained. The table gives the formula of the maximal and minimum adequate models obtained following model simplification. The sample size and the specified error structure of the model are given. The tables of coefficients from the maximal models are given as monitoring effort is not biologically informative but was included in the model to control for variation in observation intensity. The estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest are provided.

Data from 2016				
Maximal model	Number of mates obtained ~ No. days fed (nymph) + No. days fed (adult) + Monitoring effort			
Min. adequate model	Number of mates obtained ~ Monitoring effort			
Sample size	26			
Model error structure	Poisson			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	-0.42	0.6568		
No. days fed (nymph)	0.004	0.0222	0.137	0.71
No. days fed (adult)	-0.06	0.0757	2.921	0.09
Monitoring effort	0.07	0.0225	42.691	< 0.001
Data from 2015				
Maximal model	Number of mates obtained ~ No. days fed (nymph)+ Monitoring effort			
Min. adequate model	Number of mates obtained ~ Monitoring effort			
Sample size	31			
Model error structure	Poisson			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	-1.86	0.9077		
No. days fed (nymph)	0.015	0.0259	1.276	0.087
Monitoring effort	0.1	0.029	42.691	< 0.001

Table 12: Table summarising the effect of supplementary feeding of nymphs (in 2015 and 2016) and adults (in 2016), and monitoring effort on the number of mating events a male achieved. The table gives the maximal and minimum adequate models as well as the sample size and error structure for the model. The tables of coefficients from the maximal models are given as monitoring effort is not biologically informative but is included to control for variation in observation intensity. The estimate and 95% confidence interval, the test statistic, and the P value for the term of interest are provided.

Data from 2016				
Maximal model	No. of mating events achieved ~ No. days fed (nymph) + No. days fed (adult) + Monitoring effort			
Min. adequate model	No. of mating events achieved ~ Monitoring effort			
Sample size	26			
Model error structure	Negative binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	0.34	0.8296	0.792	0.428
No. days fed (nymph)	0.016	0.0398	0.598	0.439
No. days fed (adult)	-0.14	0.1255	3.744	0.053
Monitoring effort	0.12	0.0402	34.009	< 0.001
Data from 2015				
Maximal model	No. of mating events achieved ~ No. days fed (nymph) + Monitoring effort			
Min. adequate model	No. of mating events achieved ~ Monitoring effort			
Sample size	31			
Model error structure	Negative binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	-1.61	1.0452		
No. days fed (nymph)	0.03	0.0394	1.929	0.165
Monitoring effort	0.15	0.0413	56.897	<0.001

Discussion

My results show a positive relationship between mass at emergence and the number of days a nymph received the dietary supplement. A similar study, using adult *G. campestris* males also found that dietary supplementation increased body mass, even over the relatively short period of 6 days (Holzer *et al.*, 2003). Interestingly however, I do not find any change in body size (measured as the width of the pronotum) associated with dietary supplementation. If increased body size is beneficial to crickets then it would be expected that with increased resources, nymphs would invest in body size and dietary supplementation should be associated with a positive change in body size. As such an effect is not detected, it must be considered that increasing body size is not particularly advantageous. In the house cricket *Acheta domesticus*, the greatest gain in mass occurs in the last nymphal instar (Clifford and Woodring, 1990) therefore rather than investing in further growth late in development, thus increasing size, it may be more advantageous to invest in energy reserves, stored in the abdomen, which would increase mass.

That being said it is also possible that the methodology employed here is not sufficient to illicit such a response. It simply may not have been possible for differences in diet to produce differences in size over the timeframe of the experiment as crickets grow incrementally as they moult, and most of the nymphs used in this experiment experienced only one or two moults between the start of the experiment and reaching adulthood. In *A. domesticus*, Patton (1978) found no difference in mass nor length between final instar nymphs and adults suggesting that even with dietary supplementation it may not be likely

that significant divergence in size can be achieved within the time available here.

Reaching a critical mass is thought to be an important determinant in the timing of insect ecdysis (Blakley and Goodner, 1978, Nijhout, 1979, Nijhout and Williams, 1974). It was therefore expected that diet-supplemented nymphs, who attained greater mass, would emerge as adults earlier in the season than control individuals. My results suggest that there are other important factors involved in the timing of emergence beyond the simple acquisition of food. The trend for the 2015 season is that dietary supplementation leads to earlier emergence, however this trend is in the opposite direction in 2016. It is not clear from these data what other factors have contributed to the differences in the timing of emergence; potential explanations include population density, ambient temperature, and average daily rainfall. Further exploration of this effect is not within the scope of this study, however the result invites investigation into interactions between environmental factors and individual condition.

Similar to a previous study of dietary supplementation in wild crickets (Holzer *et al*., 2003), I find no significant effect of dietary supplementation on the lifespan of males. This is not an entirely surprising result as males need to invest in both reproduction and somatic maintenance so, assuming a trade-off, my results suggest that males invest additional resources into reproduction rather than longevity. In line with this, I hypothesised that diet-supplemented males, assumed to be in better condition, with greater energy reserves, would commence mating earlier than control males, on the assumption that this could allow greater lifetime reproductive success. This is found not to be the case as

there is no difference in the time between emergence and an individual's first mating event associated with dietary supplementation. I do however find a negative relationship between dietary supplementation and how soon a male leaves his burrow post-emergence. Both male and female adults roam among burrows extensively throughout the reproductive season, however, a significant period of time following emergence has been observed where individuals remain in the close vicinity of the burrow at which they emerged (Rodriguez-Muñoz, *Pers. Coms*). The reasons for this delay and what determines the decision to leave are not clear but these results suggest that it may be related to food acquisition. Moving between burrows presents risks, such as exposure to predators or adverse weather, and so the benefits must be perceived to outweigh the costs. Control males are likely exhaust the food supply available at their burrow faster than diet-supplemented males who are receiving a near-infinite supply of high-nutrient food. At this point, the benefit of obtaining a new and richer food source may be greater than the risks of moving to a new burrow. Better fed males experience a different cost-benefit structure and so delay moving to a new burrow. As dietary supplementation has no significant effect on lifespan or mating success, it is likely that the decision to move burrow, at least in the early part of the reproductive season, is driven more by food availability than the potential to obtain a mate.

Regarding the effect of dietary supplementation on investment in sexually selected traits, I find a marginally non-significant trend that the probability of a male winning a fight in 2016 increases with the positive difference in days supplemented as a nymph between an individual and his opponent. While not conclusive, there is some suggestion that dietary supplementation may affect

fighting ability in this system. As there is no apparent effect of dietary supplementation of nymphs on fighting success in 2015, this could suggest that there is some other factor that differs among years and interacts with diet. Intriguingly, I find a much less significant effect of the difference in days fed as an adult on fight outcome. Arthropod fights are, however, often settled by asymmetries in resource holding potential (Vieira and Peixoto, 2013), which can be described as a combination of morphological and physiological traits affecting strength, persistence and endurance (Lailvaux and Irschick, 2006, Mowles et al., 2011, Parker, 1974). In other insects it has been shown that dietary conditions during sexual maturation (similarly timed to supplementary provisioning in the present study) can affect future performance in agonistic interactions (Hopwood, Moore and Royle, 2013; 2014). Therefore, if there is a real effect of feeding nymphs, my result may be explained by a strength component should dietary supplementation allow individuals to produce more muscle tissue. As insects do not grow as adults, the greatest gains in muscle mass will occur as nymphs and so be affected by variation in nymphal diet, and be less dependent on conditions during adulthood. The result here is inconclusive but should not dissuade investigation of the effect of dietary supplementation and condition on contest outcomes. Aggressive interactions have been recorded here as two individuals displaying aggressive behaviour towards each other (making physical contact, flaring mandibles) with the loser being assigned as the individual who leaves the burrow area after the fight. This is a very simplified account of what is likely to be a far more complex interaction. Furthermore, the motivation of each individual is also highly variable and an important determinant of fight outcome. It has been shown in blackbirds, *Turdus merula*, that acoustic signals inform the receiver of the sender's

motivation to fight and this is likely to have an effect on receiver behaviour (Ripmeester, de Vries and Slabbekoorn, 2007). The outcome of male-male contests might therefore be determined by a number of signals, cues or behaviours which would not be detected here, such as aggressive acoustic signals and fast or small movements invisible to the camera. Many of the variables which contribute to determining a victor will undoubtedly be sensitive to individual condition, but the scope and power of this study is not sufficient to make robust claims about this effect.

I do find a positive relationship between dietary supplementation and calling effort which supports the indicator trait hypothesis. This result is consistent with Holzer *et al*. (2003) who report an approximate 10% increase in the probability of singing in food supplemented males relative to control males, across the 6-day experimental period. However, despite increasing calling effort, I find that dietary supplementation had no significant effect on the number of mates that a male obtained, nor the number of times he mated during his lifetime. This suggests that the increase in calling effort is not sufficient to increase male mating success (which would render it invisible to sexual selection) or at least that the effect is so small that I was unable to detect it.

This finding is in contrast to Holzer *et al*. (2003) who extrapolate reproductive success from the increased “attractiveness” of food supplemented males (relative to control males), measured by the number of females trapped at male-occupied burrows. However, assuming that females trapped at male occupied burrows would have mated with those males may be an inappropriate assumption as in my study population, during the 2015 and 2016 seasons, only

66% of female arrivals at male-occupied burrows resulted in mating, and so it is likely that Holzer *et al.* (2003) overestimate the effect of supplementation on mating success. The methodology employed by Holzer *et al.* (2003) may have caused further interference with the natural behaviour of the study population, leading to unreliable estimates of the effect of supplementation. To ensure correct delivery of the food supplement, males were confined to the area immediately surrounding their burrow throughout the experiment and attractiveness was estimated as the number of females trapped male-occupied burrows. This methodology makes four important assumptions which may not be appropriate; that there is little variation in reproductive behaviour between virgin and mated males, that no other factor beyond condition constrains calling effort, that mating only occurs when females arrive at male-occupied burrows, and that females assess males based only on calling song.

While caging males at their burrows ensures correct provisioning of the food supplement, enforcing male virginity means that the costs of repeated mating or variation in resource allocation between mated and virgin males cannot be considered. Allowing males to mate may be important in determining the ultimate effect of condition on reproductive success. For example, Zajitsheck et al. (2012) found that mated males lived longer than virgin males in *T. commodus*, perhaps due to the increased calling effort observed for virgin males.

Furthermore, it is assumed that if females prefer those males that sing the most then the optimal strategy should be to sing at the maximum level that condition allows (Rowe and Houle, 1996), yet condition may not be the only constraint on

singing behaviour. Acoustic signals have the potential to advertise presence to predators or parasites as has been shown in crickets (Cade,1975) and birds (Mougeot and Bretagnolle, 2000), as have a variety of other sexual signal modalities in other taxa (Godin and McDonough, 2003; Johnson and Candolin, 2017; Zuk and Kolluru, 1998). Risk of predation or parasitism can impose additional costs on calling behaviour, beyond the associated energetic costs and so the exclusion of such factors may overestimate lifetime reproductive success for vigorously calling males.

Further, the arrival of females at male-occupied burrows is not the only way by which mating occurs in this species. Male *G. campestris* are known to frequently move among burrows and often encounter females with whom they often mate (Hissmann, 1990). Indeed, in my study population, males are regularly observed to arrive at female occupied burrows and during the 2015 and 2016 field seasons, 56% of these encounters resulted in mating. Calling and searching may represent specific strategies for obtaining mates and their relative prevalence has been shown to be affected by environmental conditions (Cade and Cade, 1992). Therefore, by considering only the mating events that could be achieved with females that arrive at a male's burrow, Holzer *et al*. (2003) likely underestimate the reproductive success of at least some males and remove an important source of variation in mating success.

Finally, it is also likely that male reproductive success is determined by more than just his investment in calling. Females often have multiple methods by which they can assess potential mates as this reduces the risk of mate choice errors (Møller and Pomiankowski, 1993). For example, in the greenfinch,

Carduelis chloris, wing patches and tail patches reveal different male attributes used in mate choice (Lindström and Lundström, 2000), while in zebra finches, *Taeniopygia guttata*, beak length and song rate are both used as sexual signals (Birkhead, Fletcher & Pellatt, 1998). In crickets, while calling is considered to be a primary signal for mate attraction, mate assessment is known to also tactile and chemical cues (Loher and Dambach, 1989, Tregenza and Wedell, 1997). Therefore, while females were more attracted to greater calling effort, final mate choice decisions could be based on a number of criteria and so simply counting the number of females assumed to be attracted to a given male is not a reliable estimate of mating success.

The methodology that I have employed avoids these assumptions by allowing free movement of all individuals and estimates mating success by direct observation of mating events. It is interesting then that despite observing increased calling effort associated with dietary supplementation, there is no detectable, associated increase in mating success. This may be because variation in calling effort associated with dietary supplementation is small relative to the among-individual variation in calling effort (Figure 6). Therefore, even if a male is provided the dietary supplement for many days, he may not be the most prolific caller in the population. A second possibility is that calling is a complex energetic task and is likely influenced by a number of additional biotic factors beyond condition, such as aerobic capacity or age (Verburgt, Ferreira and Fergusson, 2011). The transmission of the signal may also be affected by abiotic conditions such as wind direction and speed, environmental noise, or the male's position in the meadow. It has been shown in crickets and other taxa that environmental conditions can interfere with the efficacy of sexual signals in

mate attraction (crickets: Schmidt *et al.*, 2014; wolf spiders: Gibson and Uetz, 2008; cichlids: Seehausen, van Alphen and Witte, 1997; sticklebacks: Wong, Candolin and Lindström, 2007). Therefore, while supplementary feeding certainly has an effect, there may be many other sources of variation impacting female response calling effort. A final explanation, is that females may have additional ways of assessing mate quality, as discussed above. Variation in female response has been found to be associated with structural components of male song in anurans (Gerhardt, 1991), birds (Ballentine, Hyman and Nowicki, 2004) and indeed crickets (Popov and Shuvalov, 1977) and females may be gathering information on potential mates that is of equal, or greater value, than his calling effort. Conversely, upon encountering a male, females may employ additional assessment methods, such as assessment of cuticular hydrocarbons (Tregenza and Wedell, 1997) to avoid making poor mate choice decisions.

The major limitation in drawing strong conclusions from negative findings in this study is the limited sample size available. Nymph movement among burrows is minimal and adult tagging occurs as soon after emergence as possible, however on a number of occasions individuals moved to new burrows before they could be tagged, resulting in potentially equivocal assignment of treatment and the exclusion of that individual from the experiment. This study has shown that it is possible to conduct wild experiments with a similar observational intensity as employed in laboratory studies with the use of video cameras, but it also emphasises the requirement of maintaining close control of experimental animals. Regrettably, the reduction in sample size has prevented the incorporation of age as an explanatory variable in this experiment.. However, the present study observes individuals throughout their adult life and dietary

supplementation did not affect lifespan or reproductive success. Therefore, the inclusion of age-related effects is not expected to change these results. This is not to say that age-related effects are not present in this population and indeed it would be of great interest to determine if individuals invest in different traits at different times according to their condition.

Furthermore, the difficulty of collecting sufficient mass determinations to allow close monitoring of variation in mass prevented a thorough investigation of the time-span over which dietary supplementation affects condition. Holzer *et al.* (2003) find that the mass of fed adults increased over a 6-day period and a similar increase in mass, associated with dietary supplementation of nymphs, is found here. However, Holzer *et* al. (2003) conducted their study over too short a time frame, and the present study lacks sufficient data to make strong conclusions about the longevity of the effect this has on condition, nor how the crickets respond when the dietary supplement is removed. Typically, where an effect of dietary supplementation of adults has been found, I have not found the same effect in response to the dietary supplementation of nymphs. This suggests that the effect of supplementation is not long lasting, however a more extensive time-series analysis of mass change would be required to properly quantify the relationship between condition and supplementary feeding.

I have demonstrated potential condition-dependent life history effects in a wild population of insects. I have shown that dietary supplementation can reveal condition-dependent differences in the expression of sexually selected traits, consistent with the indicator trait hypothesis, and that this is observable in a wild context. However, I also show that these effects can be small and emphasise

that the observation of secondary sexual traits alone may not be sufficient to infer reproductive success. The overall effect of sexual selection on adaptation remains an exciting and important question in evolutionary biology. Further such studies must consider realised reproductive success, ideally by assigning parentage across generations, rather than relying on potential reproductive success extrapolated from signalling effort. I encourage the incorporation of relatedness analyses in wild studies to obtain more accurate measures of reproductive success and provide the best understanding of the role of sexual selection in adaptation in nature.

Considerations for future dietary manipulation in wild systems

Manipulation of individual condition through dietary regimes is a powerful and popular laboratory tool. The present study, along with similar (Holzer et al., 2003, Jacot et al., 2008), have shown that this approach can be employed in the wild to good effect. Conducting rigorous experiments in the wild is a difficult task however, and future studies should be mindful of the associated challenges.

The choice of food supplement has important practical implications. The food pellet used in the present study was chosen because it was the most comparable to that used by (Holzer et al., 2003). The pellet was however, very absorbent and quickly rotted once wet. This meant that while every effort was taken to keep the pellet out of contact with the ground, feeders still had to be removed each evening and replaced each morning, as well as any time it rained. Not only is this very time consuming, it also restricts the time that the animals are able to feed. *G. campestris* forages mainly during the day and so

this was not a vital issue here, but future studies should consider the foraging habits of their system and the practical implications that this may impose.

I had intended to quantify the rate of food pellet consumption for each cricket by weighing the food pellet before placement and reweighing it upon collection. The affinity of the pellet to absorb water made accurate measurements of mass difficult, even after drying in an oven. Furthermore, video data revealed that many other invertebrates readily fed from the food pellet. It therefore became impossible to determine food consumption with any reliability and so attempts to obtain these measurements were abandoned. Should future studies wish to capture variation in consumption rates, great care must be taken to exclude other animals from feeding on the supplement. The best methods by which this could be achieved will certainly be specific to each system.

The costliest challenge in the present study was the timing of adult tagging. *G. campestris* nymphs rarely move burrow (in this study it is estimated that no more than 10% of nymphs moved to a different burrow before adulthood). Nymphs were not tagged as this would prevent ecdysis and so tagging was performed on adults only. The sample sizes available in this study were, however, drastically reduced due to failure to tag adult crickets before they moved burrow. Adult crickets began moving among burrows earlier after emergence than was previously thought and so a reliable account of individual feeding history could only be obtained for 42 out of a possible 108 individuals during the 2016 season. Future studies should be mindful of this risk and design rigorous methods of individual identification.

Chapter Three

Body size fails to predict mating success in a population of wild field crickets

Abstract

Larger individuals are often observed to be superior to smaller individuals, either in terms of mating success or fighting ability. However, broad variation in body size, within- and among species, suggests that there must be some selective constraint on body size, or that different scenarios influence optimal body size in different ways. Body size is expected to be at least partly condition-dependent, since size will be constrained by the abundance and quality of food resources the individual is able to obtain.

Field crickets are a popular experimental laboratory system in sexual selection. Numerous studies have identified associations between male traits and mating success. Frequently, large body size is among the traits associated with mating success. This raises the question of whether this observation is an artefact of the laboratory environment or whether it is equally important in nature. I used video data from a wild population of *G. campestris*, observed over ten reproductive seasons, to investigate the effect of male body size on lifespan, calling effort, mating rate and mating success, and fighting ability. I find that larger body size is associated with increased fighting ability, but that body size does not predict mating success, calling effort, or lifespan. These results suggest that increased body size may not be an important variable in this system in terms of sexual selection. My finding that being larger increases the likelihood of winning a fight is consistent with many laboratory studies. However, the importance of fighting ability in determining mating success is unclear in this species.

Introduction

From the antlers of Irish elk to the peacock's tale to fiddler crab claws, sexual selection is heralded as being responsible for some of the most exaggerated traits observed in nature. Such traits, seemingly contrary to natural selection, have captured the attention of researchers throughout the years. To this day still, understanding what factors contribute to the evolution of such traits, what constrains their development, and what maintains their variation, remains a key area of research. There is a great wealth of empirical evidence supporting the notion that in nature bigger is better, as relatively larger individuals are frequently observed to enjoy greater reproductive success. Larger individuals have been shown to attain higher ranks in dominance hierarchies (Nelissen, 1992), to win more fights in male-male contests (Elias et al., 2008, Schuett, 1997), and to be more attractive to females (Brown, 1990, Simmons, 1986a, Simmons, 1992), than their smaller conspecifics. The advantage of greater size has even been seen in sperm competition where larger sperm outcompete smaller competitors (LaMunyon and Ward, 1998, Radwan, 1996).

Should bigger always be better?

When greater size provides such an advantage, the obvious question is why is anything ever small. Without some limiting or stabilising factor, size should increase continually and the world should be filled with giants of every species. This is, of course, not the case and a number of possible explanations have been proposed. Many of these are reviewed in Blanckenhorn (2000), which considers a number of possible mechanisms, some of which I highlight here. To become a large adult, juvenile individuals may either spend more time growing or grow more quickly. Those who spend longer growing have an increased

chance of mortality before reproducing (Roff, 1980, Stearns and Koella, 1986), while those who grow more quickly may suffer a higher risk of predation owing to the increased foraging effort required to do so (Abrams et al., 1996, Fraser and Gilliam, 1992, Werner and Anholt, 1993). Furthermore, genes that promote short generation times will increase in frequency relatively rapidly, and so are always under strong directional selection. For large size to evolve, the evolutionary forces driving shorter development times must be counterbalanced.

If an individual does become a large adult, they will require more resources to sustain themselves (Blanckenhorn et al., 1995, Clutton-Brock, 1988, Clutton-Brock et al., 1985, Reiss, 1991) and will be more visible to predators and less able to evade them (Andersson, 1994). Similarly, and pertaining specifically to sexual selection, smaller males may be more agile in courtship displays and mate searching (Banks and Thompson, 1985, Blomqvist et al., 1997, Neems et al., 1998, Steele and Partridge, 1988) or, requiring less resources, be able to spend more time and energy on reproduction (Blanckenhorn et al., 1995, Jönsson and Alerstam, 1990, Schwagmeyer, 1988, Simmons et al., 1999, Taborsky, 1998). Sexual selection for large and for small males has been found to occur within the same species (Blanckenhorn et al., 1995, Serrano-Meneses et al., 2007, Travis, 1994) offering another plausible mechanism for stabilising selection acting on body size (Mason, 1964, Moore, 1990).

When stabilising selection is acting on body size we should not expect larger males to always achieve the greatest reproductive success, because large size should not be ubiquitously advantageous. As Blanckenhorn (2000) notes

however, empirical evidence of selection for smaller body size is rare. Yet since the publication of that review, an increasing number of studies are emerging showing that large body size can be a disadvantage (Berger et al., 2006, Cardillo et al., 2005, Carlson et al., 2008, Dibattista et al., 2007, Harrison et al., 2013a, Ismail et al., 2012, Locatello et al., 2016, Passow et al., 2015, Ringsby et al., 2015, Stillwell et al., 2008, Zhu et al., 2016). Furthermore, some studies suggest that body size may be irrelevant in terms of reproductive success in a number of taxa including Insects (Charlwood et al., 2002, Rashed and Polak, 2009, Shackleton et al., 2005), Reptiles (Clark et al., 2014, Lappin and Husak, 2005), and Amphibians (Croshaw and Pechmann, 2015, Fan et al., 2013).

While the ubiquity of the advantage of greater body size in sexual selection is being called in to question, it is not always obvious when greater size will be advantageous. A number of studies offer conflicting evidence as to the role body size plays in sexual selection, even in closely related species. For example, in a no-choice mating latency experiment Shackleton et al. (2005) found no relationship between male body weight and attractiveness in the field cricket, *Teleogryllus commodus*. However, female house crickets, *Acheta domesticus*, have been found to show a preference for larger males in a two-choice tournament experiment, in response to both visual and auditory stimuli (Stoffer and Walker, 2012) . Similarly, Jones et al. (2002) found that longer male Rough-skinned Newts, *Taricha granulosa,* who had higher tails enjoyed greater reproductive success than their smaller conspecifics, a finding corroborated by parentage analysis of wild-caught individuals of this species (Jones et al., 2004). Yet, in Marbled salamanders, *Ambystoma opacum*, larger males did not

sire more offspring than smaller males in experimental breeding groups (Croshaw and Pechmann, 2015).

Nevertheless, laboratory studies – particularly those of insects – frequently test various traits related to mate attraction and male-male competition and conclude that greater size does indeed confer an advantage. For example, in honey bees, *Apis mellifera*, larger males have been shown to achieve twice as many mating events as small males in aerial swarms (Couvillon et al., 2010). In the tree cricket, *Oecanthus nigricornis*, it has been found that females respond more favourably to playbacks of the lower frequency calls of larger males (Brown et al., 1996). Furthermore, a meta-analysis of 33 studies of odonates found a significant and positive overall effect of body size on both mating rate and lifetime reproductive success (Sokolovska et al., 2000). Such findings suggest that body size can play an important role in sexual selection across insect taxa perhaps even to the point that the majority of the variation in male quality that is considered to be the target of sexual selection, may be explained by variation in body size. If body size is under such strong sexual selection in insects, the important question is what mode of balancing selection is occurring to prevent males increasing in size. However, with such conflicting evidence as to the role of body size, it is equally important to first ask if the apparent importance of body size in sexual selection in insects is merely an artefact of laboratory studies. It is this question that is addressed in this chapter.

Limitations in laboratory studies

Laboratory studies, while often very insightful, are simplifications of complex natural situations. The generality of their results relies on the assumption that observations made in the laboratory predict those that would be made in the wild. As such, these studies can be vulnerable to important limitations – primarily, is an individual's behaviour in a laboratory environment indicative of its behaviour in its natural environment? Studies that have addressed this directly have often focussed on traits such as boldness, neophobia and anti-predator behaviours, and differences between wild-caught and laboratory-reared individuals have been found in a number of taxa (Rats: Barnett, 1958; Fish: Mineka *et al.*, 1980; Primates: Wright *et al.*, 2006). Furthermore, widespread evidence of individual by environmental interactions of flexible traits, such as behaviour (see Table 1 in Dingemanse *et al.* 2010), should be carefully considered when extrapolating from laboratory studies.

Laboratory experiments aim to model natural processes, and by design remove many, if not all, other factors that may affect a response. While this remains the most powerful tool available for determining causation of effect, applying these findings in the wild is akin to extrapolating a result beyond the range of available data. Once additional parameters such as resource availability, habitat heterogeneity, male-male competition, and predation risk are reintroduced, the strength of an effect may be drastically different. For example, when laboratory-derived predictions of dominance were applied to a wild population of brown trout, *Salmo trutta*, the fitness advantage of aggressive, dominant individuals over nonaggressive individuals was found to have been overestimated in a natural, heterogenous habitat (Höjesjö et al., 2002). Furthermore, Fisher et al.

(2015) showed that boldness was a repeatable behaviour within-individuals of wild-caught field crickets, *Gryllus campestris*, when tested in the laboratory. However, when these same individuals were tested in the field it was found that their laboratory scores did not predict their scores in the wild.

Another important consideration is that wild-caught individuals may not represent a random sample of the population. One might expect to capture a greater proportion of larger, bolder individuals, or those individuals who forage more often, than is truly present in the population. This is, in essence, analogous to the mechanisms of stabilising selection acting on larger individuals via increased predation risk as discussed above.

Furthermore, mating preferences have been shown to be context-dependent in a variety of scenarios (population density: Cade and Cade (1992), Hissmann (1990); seasonality: (Qvarnstrom et al., 2000); predation risk: Atwell and Wagner (2015)). When individuals are kept in artificial or stressful conditions, mating preferences may shift from what would be observed in the wild. For example, in the field cricket, *Gryllus bimaculatus*, females showed a size preference for potential mates when shelter was available, but were less selective when shelter was not provided (Simmons, 1986a). In blue tits, *Cyanistes caeruleus*, and starlings, *Sturnus vulgaris*, it has also been shown in that while neither species has an innate preference for ultraviolet or non-ultraviolet lighting, both prefer potential mates viewed in ultraviolet light than those viewed in non-ultraviolet light, independent of any attribute of that potential mate (Hunt et al., 1999, Maddocks et al., 2002). This suggests the

possibility that unnatural sensory environments may confound experiments of mate preference.

The Cricket Model

Crickets are a favoured model system for laboratory studies owing to their ease of handling and rearing, tolerance of laboratory conditions and a suite of definable behaviours. As such, they have been influential in a number of important studies of sexual selection. A non-exhaustive review of the literature appears in appendix 2a and provides a summary of 116 studies which have used crickets from 27 species as a model system for studying sexual selection in a variety of contexts. Some species appear to be overrepresented in these studies as over 50% of the papers highlighted here use one of just five species (*G. bimaculatus* (14 papers), *T. oceanicus* (14 papers)*, A. domesticus* (11 papers), *G. pennsylvanicus* (9 papers), and *G. sigillatus* (8 papers)). One might suppose that this is due to the ease with which these species can be reared under laboratory conditions as almost 90% of the studies included in this table are lab-based, but it should not be assumed that these species can represent all Gryllidae. Of the studies reported here, 34% directly test the effect of body size on mating or reproductive success, or on a sexually selected trait. Of these 40 studies, 24 find body size to have an effect (60%). Qualitatively, there does not appear to be an obvious pattern to whether a study finds body size to have an effect based on the dependent variable investigated or the species used in the study.

Even in as well-established a model system as Gryllidae, care must be taken to consider the extent to which laboratory findings reflect natural systems. To

investigate the ultimate role of body size in sexual selection in nature, it is important to be able to observe responses to variation in body size in a natural setting, but maintain the monitoring intensity of a laboratory study. The WildCrickets project has been monitoring a population of field crickets, *Gryllus campestris*, in this way for 11 years using a network of video cameras. This allows wild insects to be intensively monitored in their natural habitat, avoiding any confounding effects that a laboratory environment may impose. Using video and morphological data I have investigated fundamental questions about body size and sexual selection in a natural and wild system.

This investigation is in three parts, each representing basic questions addressed in laboratory studies. Firstly, I ask if larger males enjoy any sexually selected advantage in this population. If body size represents a condition dependent trait, or indeed improves the survival of the male, then it is expected that females should prefer larger males due to the genetic benefits that can be gained for their offspring. In either case, body size would influence sexual selection and so to determine if this is the case in a wild system I investigate the role of body size in determining both the number of mates obtained by each male and his mating rate throughout adulthood.

Secondly, I investigate the relationship between body size and investment in an energetically demanding secondary sexual trait, male calling song. This is a condition-dependent method of mate attraction in field crickets (Holzer et al., 2003, Judge et al., 2008, Ryder and Siva-Jothy, 2000, Scheuber et al., 2003, Tregenza et al., 2006, Wagner and Hoback, 1999) and greater investment in calling is expected to lead to greater mate attraction (Holzer *et al.* 2003).

Condition-dependence could lead to males who are both large and invest heavily in calling (high quality males who are able to gather sufficient resources to do so) or lead to trade-offs where one can invest in reproduction through calling or in, perhaps, survival through size. I therefore investigate the relationship between body size and calling effort to determine if this song represents a signal of size or is subject to a trade-off between somatic maintenance and reproduction in the wild. I further investigate the effect of body size in predicting the winner in male-male agonistic contests. While evidence to the effect of fighting success on reproductive success in crickets is mixes, body size is often reported to correlate with fighting success in laboratory studies (see Arnott and Elwood, 2009). I here investigate if this is true in the wild also by investigating naturally occurring fights between males.

Finally, I assess if females express a preference for larger males and ask if the latency to mate is an attribute of males or something that is independently determined by the female alone. Mating is costly (Daly, 1978; Dawkins and Guilford 1991), and so females must assess males and decide whether or not to mate. Theory predicts that females should mate sooner with the most attractive males and be more hesitant with less attractive males (Cotton, Small and Pomiankowski, 2006), thus latency to mate (the time between introduction and the commencement of copulation) is a useful estimate of male attractiveness. If body size is an important determinant of the attractiveness of wild male *G. campestris*, then it is expected that females will mate sooner with larger males than smaller males.

Methodology

The WildCrickets Field Site and Behavioural Monitoring

The following experiment was conducted at the WildCrickets field site in Asturias, Spain during the 2015 and 2016 reproductive seasons. The study population of field crickets, *G. campestris*, which have been established here for over 40 years (Rolando Rodriguez-Muñoz *pers. comms*), occupy an 800 m^2 area of relatively flat grassland. Crickets create and take refuge in burrows dug into the substrate and it is expected that the vast majority of cricket behaviour takes place in the immediate vicinity of these burrows. Each year, these burrows are detected manually by researchers and identified by a unique number. One of up to 130 video cameras (VivoTek, Taiwan) are deployed at each burrow to record cricket behaviour. The video cameras are deployed prior to crickets emerging as adults and continue recording throughout the reproductive season until the last individual dies. The cameras are equipped with day-night capability and so run continuously throughout this time.

To enable identification of individual crickets, adults are trapped at their burrows, using bespoke traps (see crickettrapping.wordpress.com for details), from two days post-emergence and fitted with a PVC tag. The tag is glued to the pronotum using cyanoacrylate glue and bears a 1-2 character code unique to each individual, which is easily read during video playback. During trapping morphological measurements are also made; specifically individual mass using a precision balance (SNUG II-300, Jadever, Taiwan) and pronotum width. Pronotum width is used as a proximate measure of body size and is measured

from top-down photographs of the pronotum made using a digital camera (PL210, Samsung, South Korea) connected to a dissecting microscope (S6E, Leica Microsystems, Germany). Pronotum width is measured using FIJI image analysis software (Schindelin et al., 2012), and each measurement is calibrated to the character height of the ID tag (see Figure 4). Three photographs of each individual were made, with individual being allowed to slightly adjust its position between photographs, so that an average measurement per individual could be used. This accounts for among-individual error caused by each cricket's position relative to the camera. Following a brief period of recovery, crickets were returned to the burrow from which they were captured. Traps were left in burrows during capture to prevent the burrow being taken over by other individuals.

Data on cricket behaviour was extracted from the recorded videos by manual playback. Playback was conducted in a three-step process: first to detect video sections which contained cricket activity, secondly to extract behavioural data from the video, and finally to check and rectify inconsistencies in the behavioural record. During the second stage, any of 19 key events were recorded (See tables 1 and 2) along with the burrow these events occurred at, the date and time of the event, and the ID's of the crickets involved.

For a full account of the methods highlighted above please refer to Chapter One: General Methodology.

Quantification of male calling song

Male calling effort was estimated through additional video review. The first 10 minutes of each hour that a male cricket was observed by a camera was observed in 1-minute intervals. A binary response was recorded for calling behaviour if the male was observed to call at any time during this 10 minute observation period. This allowed calling effort to be estimated as a proportion of the number of observation periods a male was observed to be singing relative to the number of observation periods for that male. Calling behaviour could be determined from the video recordings due to the raised position of the wings which is distinct to this behaviour and does not coincide with any other activities. A full account of this methodology is given in Chapter One: General Methodology.

Effect of size on mating success

Male mating success was investigated in two ways: analysing the number of unique mates that a male obtains across his lifetime, and also each male's observed mating rate. The effect of body size on the number of mates a male obtained was analysed using a Generalized Linear Mixed Model (GLMM) with a Poisson error structure and a square root link function. The square root link function was adopted instead of the default log link function as the former was determined to better fit the data based on visual inspection of the model residuals. The maximal model contained body size and adult lifespan (number of days from emergence to death, inclusively) as main effects, along with the interaction between these variables, and with the year of the field season included as a random factor to control for variation among generations. The data were subset to include only those males whose emergence date and date

of death could be assigned to within one day to avoid misestimating lifespan for less observed individuals. Lifespan was included in the model primarily to account for variation in the number of mates caused by differences in male longevity as even if larger males do obtain more mates, a large male who is predated after two days is not expected to obtain many mates. Including lifespan as a fixed effect also allows potential differences in life history strategies to be detected. A non-significant result of lifespan would suggest that short-lived males are obtaining as many mates as longer-lived males who would be expected to be more successful, thereby inviting investigation of different mating strategies. A point estimate of the dispersion parameter was obtained using R code provided by Harrison (2014), and indicated that the model was not overdispersed.

Male lifetime mating rate was calculated by counting the total number of mating events a male was observed participating in and dividing this by the total time that individual occupied a burrow that was under observation by a camera (monitoring effort). This value was expressed in days as a decimal value such that a value of 0.3 represented approximately 8 hours while a value of 2.5 would represent 56 hours. Only males who had at least 24 hours of monitoring time (i.e. a monitoring effort of >1) were included to avoid less reliable estimates obtained for individuals who were rarely monitored. The effect of body size on lifetime mating rate was analysed using a GLMM with a gamma error structure. The gamma model was used as it was found to better fit the data than a log-normal model in terms of homoscedasticity and normality of residuals. Body size was the only fixed effect included in the model as lifespan was highly

correlated with monitoring effort. Year of field season was included as a random factor.

Effect of body size on lifespan

The relationship between lifespan and body size was investigated to determine if larger individuals were expected to live longer, thereby causing potential variance inflation in analyses including both size and lifespan. The data were subset to include only males for whom a reliable estimate of lifespan was available, as noted above. Lifespan data did not fit the Poisson distribution as the variance in this data was considerably higher than the mean. The effect of body size on lifespan was therefore analysed using a GLMM with a negative binomial error structure. This error structure allows variance to be included as an additional parameter, allowing it to vary from the mean. Size was the only fixed effect included in the model, although the year of field season was included as a random factor.

Effect of body size on fighting success

To investigate the effect of body size on the likelihood of winning a fight, the variable Δsize was calculated by subtracting the body size of the invading male, the one who arrived at an occupied burrow, from the body size of the resident male, the one who was occupying the burrow. This was done to account for individual-level effects on fight outcome as in dyadic interactions such as this, it is the pair rather than the individual that is the independent unit of measurement. It is important to account for this as an individual's ability to win a fight is not independent of the ability of the opponent, nor are the future fights of a given individual independent of the current fight, as the outcome of one fight

can influence performance in the next (see Hsu & Wolf, 1999). Only the first fight between any pair of competitors is used in the analysis, regardless of whether or not they switch roles in subsequent fights. In this analysis of fighting success in relation to body size, the focal individual is the resident cricket and Δsize represents the difference in size between resident and invader. Therefore, large positive values indicate that the resident was considerably larger than the invader while large negative values indicate that the resident was much smaller than the invader. Values close to zero indicate little difference between the individuals.

Aggressive behaviour begins very rapidly when two individuals of the same sex meet. Fighting was considered here to be any aggressive behaviour including antennation, mandible flaring, and physical wrestling. A cricket was considered to have lost a fight if it was the first individual to leave the burrow vicinity following the fight, within five minutes of the fight starting. Fights were rarely observed to last longer than five minutes and in the very infrequent cases when neither cricket left the burrow vicinity within this time, the fight was considered inconclusive and was excluded from the analysis. The analysis was therefore performed on a total of 522 fights between unique combinations of 228 males.

The effect of body size on the likelihood that an individual would win a fight was analysed using a generalized linear model (GLM) with a binomial error structure. The dependent variable, fight outcome, was a binary variable indicating if the resident male won the fight and was modelled as a function of Δsize.

Effect of body size on calling effort

The relationship between male body size and investment in calling effort was investigated by creating a matrix variable containing the number of sample periods in which a male was observed to sing and the total number of times that male was sampled across his lifespan. Many packages in R allow such a variable to act as a single response variable in linear models that use a binomial error structure. This allows the model to estimate the effect of predictor variables on the proportion of positive scores in the response variable while accounting for the reliability of the estimate for each observation.

The effect of body size on calling effort was first analysed using a GLM with a binomial error structure however this model was overdispersed (estimated by point estimate using R code from Harrison (2014)). The data were therefore reanalysed using a Hierarchical Generalized Linear Model (HGLM) with a beta-binomial error structure following the method of Lee and Nelder (1996). This method has been shown to be effective in accounting for overdispersion in binomial data (Harrison, 2015) by drawing binomial probabilities from the beta distribution, specifying shape and scale parameters for individuals.

Effect of body size on male attractiveness

The first step to determine if male attractiveness was a function of body size in the wild was to ascertain if females were more or less likely to mate with a male depending on his body size. This was done by calculating each males' mating success; the number of mating events achieved by each male, relative to the number of times he encountered a female. A GLMM with a binomial error structure was fitted with the number of mating events and number of female

encounters as a response variable following the method outlined above for calling effort, and body size was fitted as a fixed effect. Year was again included as a random factor.

Among the most common methods of estimating male attractiveness in laboratory studies of species with female choice, is mating latency. Females are presented with a single male and the time interval between introduction and the commencement of mating is recorded. It is assumed that females will commence mating more rapidly with more attractive males (Cotton, Small and Pomiankowski, 2006) and this method has been shown to be reliable in a laboratory context (Shackleton et al., 2005). In the wild however, the environment is far more variable and a female's motivation to mate may depend on many factors not related to the attractiveness of an available male, leading to overestimation of the male's influence on mating latency. To address this uncertainty, I first determined if variation in mating latency can be attributed to any aspect of males in this system, or if mating latency is entirely a property of females.

Mating latency was calculated from video data as the interval of time between a male and female meeting at a burrow and the female mounting the male. The distribution of mating latencies was heavily right-skewed with 63% of mating events occurring within five minutes of the male and female meeting. The other 37% of latencies ranged between 6 minutes and 43 hours. Mating events were therefore classified as either fast (≤5 minutes) or slow (>5 minutes) mating events. A GLM with a binomial error structure and male identity fitted as a factor was used to determine if variation in mating latency could be explained by the

identity of the male involved. If mating latency is a property entirely of females then the distribution of fast and slow mating events among males would be random. If the model reported a significant effect of male identity however, this would indicate that fast and slow mating events did not occur randomly but were somehow structured by the male involved. The mechanism by which this would occur is not considered here, the analysis only confirms if mating latency is affected by any aspect of males in a wild setting.

It was next considered if variation in mating latency could be explained by variation in male body size. Mating latency remained classified as fast or slow mating events as above and the data was subset to include only those males who mated at least six times. This was done to exclude rarely mated males who may provide unreliable estimates of their mating latency, but preserve a suitable sample size. As above, the number of fast and slow mating events for each male was fitted as a response variable in a GLM with a binomial error structure, with body size fitted as a fixed effect.

Results

Effect of size on mating success

Male pronotum width, across all observed reproductive seasons ranged between 6.57 mm and 9.28 mm, with a mean ± SE of 7.82 ± 0.027. Body size was found to have no significant effect on the number of unique mates that a male obtained (GLMM: n = 250, $\chi^2 = 0.44$, d.f. = 1, P = 0.51, Table 13). Lifespan was included in this model and did have a positive effect on the total number of mates obtained (GLMM: n = 250, $\chi^2 = 180.89$, d.f. = 1, P < 0.001, Table 13, Figure 11), however lifespan could not be predicted from body size (GLMM: n = 250, $\chi^2 = 0.0097$, d.f. = 1, P = 0.76, Table 14) in an additional GLMM fitted with a negative binomial error structure. Body size had no significant effect on the observed mating rate of males (GLMM: n = 247, $\chi^2 = 3.4$, d.f. = 1, P = 0.65, Table 15).

Table 13: Table summarising the analysis of the effect of body size and lifespan on the number of mates a male obtained, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the minimum adequate model is given and provides the model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value.

Maximal model	Number of mates obtained ~ Body size * Lifespan + (1| Year)			
Minimum adequate model	Number of mates obtained ~ Lifespan + (1| Year)			
Sample size	250			
Model error structure	Poisson			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	X^2	P
Intercept	3.86	0.3728		
Lifespan	1.86	0.2575	180.89	< 0.001

Table 14: Table summarising the analysis of the effect of body size on lifespan, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as body size was found to not have a significant effect on lifespan. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest are provided.

Maximal model	Lifespan ~ Body size + (1|Year)			
Minimum adequate model	Lifespan ~ 1 + (1|Year)			
Sample size	247			
Model error structure	Negative binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	3.58	0.0969		
Body size	-0.007	0.0278	0.07	0.791

Table 15: Table summarising the analysis of the effect of body size on the number of mating events a male achieved, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as body size was found to not have a significant effect on the number of mating events achieved. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest are provided.

Maximal model	Number of mating events achieved ~ Body size + (1|Year)			
Minimum adequate model	Number of mating events achieved ~ 1 + (1|Year)			
Sample size	247			
Model error structure	Gamma			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	-0.7	2.2234		
Body Size	0.27	0.2824	3.398	0.065

Effect of size on fighting success

The likelihood of a given male winning an aggressive interaction increased with increasing positive differences in size relative to the opponent (GLM: $\chi^2 = 43.99$, d.f. = 1, $P < 0.001$, Table 16, Figure 12).

Table 16: *Table summarising the analysis of the effect of difference in body size between competitors on the likelihood the resident male would win. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the minimum adequate model is given and provides the estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value.*

Maximal model	Fight outcome ~ Difference in body size between rivals			
Minimum adequate model	Fight outcome ~ Difference in body size between rivals			
Sample size	523			
Model error structure	Binomial			
Table of Coefficients from the minimum adequate model				
	Estimate	95% CI	X^2	P
Intercept	-0.55	0.1869		
Difference in body size between rivals	1.06	0.3324	43.994	< 0.001

Effect of size on calling effort

There was no detectable effect of body size on the likelihood that a male would be singing during an observation period (HGLM: n = 335, t_{325} = 1.53, P = 0.13, Table 17) suggesting that variation in calling effort among males cannot be explained by variation in body size.

Table 17: Table summarising the analysis of the effect of body size on calling effort, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as body size was found to not have a significant effect on calling effort. The model estimate and 95% confidence interval, the test statistic, and the P value for the term of interest are provided.

Maximal model	Proportion of time spent calling ~ Body size + (1|Year)			
Minimum adequate model	Proportion of time spent calling ~ 1 + (1|Year)			
Sample size	335			
Model error structure	Hierarchical GLM			
Table of Coefficients from maximal model				
	Estimate	95% CI	t	P
Intercept	-3.01	1.6693		
Proportion of time spent calling	0.16	0.1070	1.528	0.127

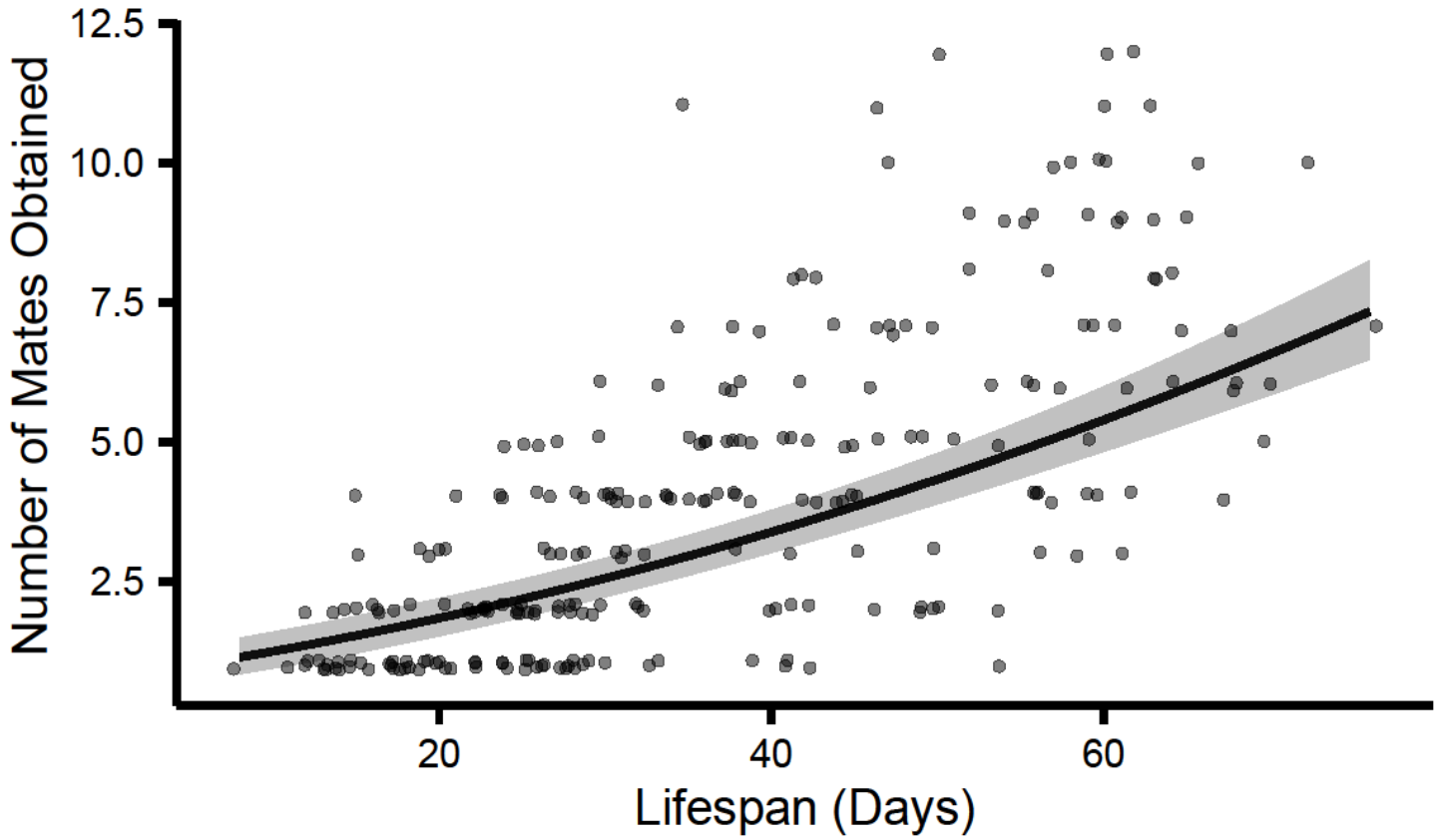

Figure 11: Plot showing the relationship between Lifespan (measured as the number of days between emergence and death, inclusively) and the number of mates obtained per male G. campestris in the reproductive seasons between 2006 and 2016 (excluding 2014). The line shown is the prediction line obtained from a poisson GLMM, and the shaded area represents the 95% confidence interval. Each data point represents a single male and transparency is applied to points to reveal overlapping data and so shades do not represent different groups.

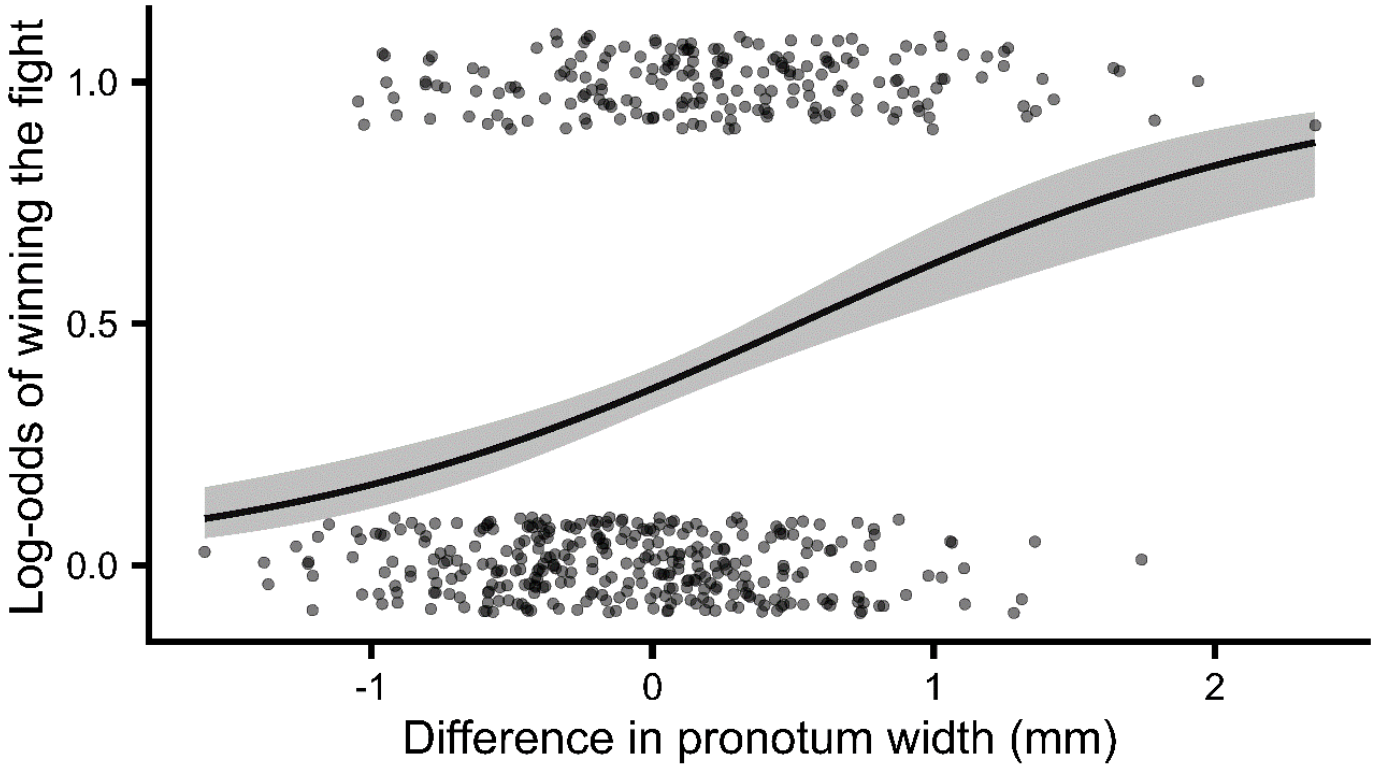

Figure 12: Plot showing the change in the log-odds of a G. campestris male winning a fight in relation to the difference in pronotum width (a proxy for body size) between the focal male and his opponent, for fights occurring in the reproductive seasons between 2006 and 2016 (excluding 2014). The line shown is the prediction line from a GLM and the shaded area represents the 95% confidence interval. While resident crickets are considered the focal individual to calculate difference in pronotum width and log-odds of winning, each point represents a fight between two males. Transparency has been used to reveal overlapping data and different shades do not represent different groups.

Effect of size on male attractiveness

Body size had no significant effect on either the likelihood that a female would choose to mate with a given male (HGLM: $t_{272} = 1.4$, $P = 0.16$, Table 18) nor on the likelihood of a male mating within five minutes of meeting a female (GLM: $\chi^2 = 0.75$, $P = 0.39$, Table 19). In a separate binomial model, Male ID was found to have a significant effect on latency classifications (GLM: $\chi^2 = 304.07$, d.f. = 218, $P < 0.001$) indicating a significant departure from the null hypothesis that the occurrence of fast or slow mating would be randomly distributed among and within males. This therefore suggests that some male traits do have a role in determining mating latency, however body size is not one of them.

Table 18: Table summarising the analysis of the effect of body size on mating success rate of a male, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as body size was found to not have a significant effect on mating success rate. The model estimate and 95% confidence interval, the test statistic, and the P value for the term of interest are provided.

Maximal model	Male success rate ~ Body size + (1|Year)			
Minimum adequate model	Male success rate ~ 1 + (1|Year)			
Sample size	282			
Model error structure	Hierarchical GLM			
Table of Coefficients from maximal model				
	Estimate	95% CI	t	P
Intercept	-1.50	1.7144		
Body size	0.15	0.2144	1.397	0.164

Table 19: Table summarising the analysis of the effect of body size on mating latency, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as body size was found to not have a significant effect on mating latency. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest are provided.

Maximal model	Proportion of short mating latencies relative to long mating latencies ~ Male body size			
Minimum adequate model	Proportion of short mating latencies relative to long mating latencies ~ 1			
Sample size	127			
Model error structure	Binomial			
Table of Coefficients from Maximal model				
	Estimate	95% CI	X^2	P
Intercept	1.503	1.5982	1.719	0.087
Male body size	0.15	0.2051	0.746	0.388

Discussion

Is size under directional selection in the wild?

The results reported here suggest that body size is not a useful predictor of mating success in this species, in the wild. I find no evidence of a significant relationship between body size and mating success – neither in the total number of mates obtained, nor male mating rate. Additionally, mating latency observations suggest that while there is some repeatable attribute of males that affects mating latency, this attribute is not body size.

It is clear that in nature the net effect of selection on body size is stabilising, for if there were continual directional selection on body size, populations would be driven toward greater and greater individual size. Yet, this net stabilising selection, paradoxically, is rarely reflected in the results of laboratory studies. It must therefore be considered that the apparent importance of body size for male mating success may be an artefact of laboratory conditions, in as much as my results indicate that in the wild, additional sources of selection appear to balance the directional selection observed in the laboratory.

By design, laboratory experiments investigate specific sources of selection on a trait by the standardisation of other ecological and environmental variables such as age, ambient temperature, predation risk, and competition (among others). It appears however that once the full suite of natural variation is reintroduced, then the commonly reported effects of body size on mating success are lost. Attention must therefore be turned to examining what factors are inhibiting size-dependent mating success in natural systems.

Are ecological trade-offs limiting size in nature?

A possible explanation as to why size-dependent variation in mating success is not detected in this population (and potentially other wild systems (Hughes et al., 2013, Lappin and Husak, 2005, Sullivan, 1983)) is that when the full richness of the natural environment is present, trade-offs occur between body size and other important naturally and sexually selected traits. The acquisition or maintenance of greater body size is expected to impose some cost, which may negatively impact the development or performance of other sexually selected traits; be these energetic costs, development time, foraging requirements, life history costs such as increased predation risk, or mechanical and physiological constraints (see Blanckenhorn (2000) for a review of mechanisms). The inclusion of such trade-offs may alter the relationship between body size and mating success, potentially reducing the effect of body size, and thereby explaining why studies conducted in natural systems fail to detect a positive effect of body size on mating success. Here, two potential sexually selected traits have been investigated in relation to body size, calling effort and fighting ability, in an attempt to detect such ecological trade-offs.

Calling effort is an energetic trait used by males to attract females and it has been shown that increased investment in calling behaviour is associated with female preference (Holzer et al., 2003, Simmons, 1986b). A positive relationship between body size and calling effort would indicate that each trait is linked by some underlying aspect of quality i.e. the individual is inherently “good” and so is both large and able to invest more in singing. Such an effect could result from an increased ability to acquire and utilise resources (**Chapter 1,** (Holzer et al., 2003)). Alternatively, a negative relationship between body size and calling effort suggests a

trade-off; the investment in body size prevents equal investment in calling effort and so an individual can be large or sing a lot, but not both. The present study finds no evidence for either relationship however, suggesting that body size and calling effort are not linked and may experience quite independent selective forces. It is therefore unlikely that a trade-off between body size and calling effort explains the lack of an effect of body size on mating success in this system.

Perhaps unsurprisingly, the larger cricket is found to be the more likely victor in male-male contests than the smaller individual. This suggests that instead of there being a trade-off between body size and fighting ability, there is a positive relationship whereby greater size confers an advantage. However, as body size has been shown above to have no significant effect on mating success, it appears that fighting ability is not a sexually selected trait in this system – males that are good at winning fights do not have greater mating success. There might be post-copulatory differences in success among males, which our measures of mating success fail to capture, but investigating this possibility would require parentage analysis which was not available here.

Why does fighting success not increase mating success?

While greater body size appears to improve fighting ability it does not increase mating success, suggesting that winning fights does little to improve a male's mating success. It has been shown in a number of systems that observation of male-male contests can affect female mate choice (Aquiloni et al., 2008, Aquiloni and Gherardi, 2010, Berglund and Rosenqvist, 2001, Doutrelant and McGregor, 2000, Ophir and Galef, 2003, van Breukelen and Draud, 2005), however there is mixed evidence for

this in crickets (Loranger and Bertram, 2016, Nelson and Nolen, 1997, Savage et al., 2004, Shackleton et al., 2005). It was not practical to investigate the effect of fighting ability on male attractiveness in this system as it would require observation of many fights where a female was present and subsequently mated with either male, in the absence of any courtship song, which is a rare scenario. In any case, it is not expected that such a preference exists in this population. If it were true that females preferred males whom they had witnessed winning fights, we should expect females to delay mating until they had witnessed a potential mate fight so as to minimise the risk of mating with an inferior mate. This would lead to females being present during more fights and preferentially mating with the larger, winning male. Ultimately this should lead to a relationship between size and mating success which is not detected here.

It may be a simpler explanation that body size is instead under natural selection, driven at least in part by the advantage that greater size provides in male-male contests. Individual fitness is likely to decrease with time spent away from a burrow as the risk of predation and exposure to adverse environmental conditions increases. This may be costly enough to drive directional selection of body size so that one may be better able to usurp a burrow or resist eviction, however no formal quantification of these costs has been found for crickets. Directional selection on body size may then be stabilised by selection favouring other traits that trade-off against body size development, suggesting a misalignment of natural and sexual selection.

The present study supports a growing body of evidence that finds that directional selection on body size by sexual selection is not necessarily the norm in wild animals

(Charlwood et al., 2002, Clark et al., 2014, Croshaw and Pechmann, 2015, Fan et al., 2013, Lappin and Husak, 2005, Rashed and Polak, 2009, Shackleton et al., 2005). Here I have demonstrated that body size is not an important determinant of mating success in *G. campestris* in the wild and that previous, conflicting results may be due to laboratory conditions. Further work will be required to ascertain if such an explanation holds true in other taxa. Nevertheless, the role of body size as a driving force behind variation in mating success should not be treated as a universal rule, nor should greater size be automatically assumed to be advantageous. Further work is required to understand what factors limit directional selection on greater body size in the wild and cause the differences seen between lab and field studies. Importantly, wherever possible, further investigation should be conducted under natural conditions before any firm conclusions as to the operation of sexual selection on body size in nature are drawn.

Chapter Four

Male mate-searching tactics are affected by context but not condition in a population of wild crickets

Abstract

A male's fitness depends on his ability to obtain fertilisations, and males may have multiple routes by which this can be achieved. Some tactics for obtaining mates may be more favourable in some contexts than others. Similarly, as many traits related to mating success are condition-dependent, some tactics may only be available to high quality males and so individual condition may determine which strategy is adopted.

Field crickets have been observed to engage in two distinct mate searching tactics; signalling and searching. Signalling males call from burrows to attract females to mate with, while searching males roam silently and intercept females by repeatedly visiting burrows. Previous studies in field crickets have found variation in mate searching tactics is related to population density. I investigate whether this effect occurs in this system by comparing investment in male calling effort among nine reproductive seasons which naturally varied in population density. My results support previous studies, finding that males spent more time calling when population densities were smaller, thereby supporting the hypothesis that mate searching tactics are mediated by context.

As calling effort is condition-dependent, it may also benefit low-condition males to avoid being out-competed by superior males, and hence to favour searching over calling. I compare calling effort and movement of males from nine reproductive seasons but find no evidence of such a trade-off. Instead I find that males who sing the most also move burrows more often, indicating that calling and movement are mediated by some common underlying factor and that the choice of tactic is not condition-dependent.

Introduction

The fitness of a male depends on his ability to obtain mates and fertilisations. This has driven the evolution of a spectacular variety of traits and tactics to secure mating success; from elaborate and costly signals that promote attraction, to aggressive intrasexual contests that exclude rivals, to the meticulous maintenance of nest sites and territories.

In many systems however, males have multiple routes by which they can obtain mates or fertilisations (Gross, 1996). Much research has been conducted on alternative reproductive tactics and has revealed a variety of methods by which males of the same species might maximise their mating success (see Taborsky *et al.*, 2008). In crickets, it has been reported that males can adopt a calling strategy, where they remain at burrows and produce the characteristic song to attract females to mate from afar. An alternative searching strategy has also been observed where males remain silent and move among burrows to encounter females (Cade, 1979). Moving and calling are not mutually exclusive behaviours (Cade and Cade, 1992) – a male is not expected to only call or only move - however these activities are not known to be performed simultaneously and each have the potential to increase the male's chances of encountering a female.

Many traits relating to male mating success are, among many taxa, condition-dependent (for a review see Cotton et al. (2004)). Morphological signals require allocation of resources during development (Simmons and Emlen, 2006), acoustic signals require the expenditure of substantial energy reserves (see Ophir et al. (2010)), and increased ejaculate production requires the diversion of resources from

maintenance (Simmons et al., 2017). Everything comes at a cost and resources are finite therefore males must choose which tactic to invest in, resulting in trade-offs between tactics. While many factors may determine which tactic a male ultimately follows, they may be able to strategically adjust investment in one approach or another depending on (1) environmental context, or (2) their own condition.

Environmental Context

Previous studies suggest that males adjust their reproductive behaviour in response to population density (De Boer, 1981, Greenfield and Shelly, 1985, Jirotkul, 1999a). In crickets, it has been possible to conduct such studies on wild populations in their natural environment. Two such studies have investigated context-dependent mating behaviours in the field crickets, *Gryllus integer* and *G. campestris*, and found evidence that at high population densities males invest less time in calling and move more among burrows, while at low population density males move less and invest more in calling behaviour (Cade and Cade, 1992, Hissmann, 1990).

Cade and Cade (1992) detected this pattern in a population of *G. integer* across six years and concluded that the pattern was the result of increased costs of defending a burrow associated with greater population density. Males sing from just outside their burrow and both males and females use burrows as a refuge from predators and adverse weather conditions. Cade and Cade (1992) suggest that at high population densities there is a greater pay-off from abandoning burrow defence and switching from the calling tactic to the searching tactic, while at low population densities, when females are less likely to be encountered, it is beneficial to persist with the calling tactic despite the cost of burrow defence. This pattern is supported in

simulation studies based on this system (Rowell and Cade, 1993, Walker and Cade, 2003). Hissmann (1990) found a similar pattern in a population of *G. campestris* over a single breeding season. The proportion of males that were singing was lower earlier in the reproductive season when population density was high, but increased as the season progressed and population density declined due to natural mortality.

In each of these cases however, the mechanism that underpins the effect is not very clear. The results of Cade and Cade (1992) could as easily be explained by the presence of a parasitoid fly known to use male *G. integer* as a host and whose larvae are fatal to inoculated males. The flies use male calling to locate hosts thereby indirectly increasing the cost of calling. At high population densities, this cost outweighs the cost of remaining silent (which reduces the chances of finding a mate) as females are more likely to be encountered by chance. At low population densities, when females are less likely to be encountered by chance, the cost of remaining silent outweighs the cost of parasitism and so calling has a net benefit (Cade, 1975). Thus, one observes higher levels of calling at low population densities, and lower levels of calling at high population densities. *G. campestris* is not known to experience such parasitism and yet Hissmann (1990) detects the same pattern of calling versus movement behaviour found by Cade and Cade (1992). However, Hissmann's study was conducted over a single reproductive season with an initially high population density that declined as the season progressed. Time is therefore a potentially confounding effect and as such two factors must be considered before these results can be fully accepted. Firstly, the study does not account for age-related effects on male behaviour and it has been found that calling behaviour is age dependent in *G. campestris* (Jacot et al., 2007) and in other Gryllid species

(Fitzsimmons and Bertram, 2011, Judge et al., 2008, Verburgt et al., 2011). Secondly, by using the proportion of males singing and moving as the measurement of behaviour, the study is vulnerable to selective disappearance. If calling behaviour and other sexually selected traits are condition-dependent, then those individuals who sing the most are expected to be in better condition and so expected to live longer. Therefore, one could expect to find the same pattern in the proportion of calling males as reported by Hissmann (1990) as a result of poorer condition males, who sing less, dying earlier in the reproductive season than those who sing the most.

Nevertheless, it is intriguing that similar patterns are detected in different species, under different circumstances. Given the confounding effects present in each study it is difficult to speculate as to potential mechanisms, even if it does seem likely that the effect is true.

Individual Condition

In a number of species, males have been found to adjust their investment in intrasexual agonistic interactions by assessing the condition of their opponent relative to their own condition and either escalating the contest or withdrawing depending on their perceived likelihood of winning (Abrahams et al., 2005, Clutton-Brock and Albon, 1979, Molina-Borja et al., 1998, Mowles et al., 2010, Sullivan and Walsberg, 1985, Weary et al., 1991). It may be possible for a similar situation to arise in cases where a condition-dependent trait, that is detectable by all individuals in the vicinity, is used to attract females. Males may be able to perceive their own condition, relative to their rivals, and reduce their investment in this strategy by switching to an alternative tactic, should the potential pay-offs appear better.

Certainly, behavioural plasticity in mating tactics in response to rivals has been shown in other contexts, but this remains a relatively underdeveloped area of research (Bretman et al., 2011)

It has long been established that calling behaviour by male crickets is condition-dependent (Holzer et al., 2003, Judge et al., 2008, Ryder and Siva-Jothy, 2000, Scheuber et al., 2003, Tregenza et al., 2006, Wagner and Hoback, 1999). Calling is a very energetically demanding behaviour and places a great cost on males (Hoback and Wagner, 1997, Mowles, 2014). It has been shown that individual condition can be manipulated through dietary supplementation or restriction in crickets, both in the laboratory (Hunt et al., 2004, Judge et al., 2008) and in the field (**Chapter 1,** Holzer *et al.*,2003). However, increased calling effort, as a result of improved condition, does not appear to increase mating success for diet supplemented males (see **Chapter 1**, but see also (Holzer et al., 2003)). There are a number of possible explanations for this, including a strong genetic component for calling behaviour, multi-modal signalling, or limitations in the power of the study. However, another potential explanation is that poorer condition males may be able to assess the quality of nearby calling males and adjust their own behaviour in response. Males, upon determining themselves to be outcompeted by surrounding males may either cease calling and adopt a searching strategy instead (thereby freeing themselves from costly competition that they do not expect to win) or move to an area where competition is less intense. In either case, one would expect to find higher quality males investing more time in calling than in movement, while lower quality males move more and call less. A more mobile tactic may then allow lower quality males to

increase their mating success beyond what they could achieve if they attempted to compete acoustically.

Testing context-dependent and condition-mediated mating behaviours in the wild

The WildCrickets project has been deploying video cameras to intensively monitor a wild population of field crickets since 2006. Using behavioural data obtained from nine discrete generations of this population I aimed to determine whether male field crickets could alter their reproductive behaviour in relation to environmental context or their own condition. I ask firstly if there is a negative relationship between calling effort and amount of movement to suggest the existence of alternative behavioural strategies. If males are strategically adopting different mate searching tactics then calling males are expected to move less, while searching males are expected to call less. The present study does not consider the mechanisms by which males switch strategies but rather seeks to uncover the relationship between these behaviours. A negative relationship suggests that, for whatever reason, there is a trade-off between these behaviours while a positive relationship suggests that engagement in both behaviours may be underpinned by a common, perhaps condition-dependent, mechanism. I ask also if engagement in either activity is associated with a survival cost. If it is found that either behaviour is associated with a decrease in lifespan then this would suggest that individuals engaging more in this behaviour may be favouring reproduction over survival, thus lending further evidence to the existence of these behaviours as alternative mate searching tactics.

To address the uncertainties regarding previous studies investigating the role of population density (Cade and Cade, 1992; Hissmann, 1990) on mate searching tactics I observe the amount of calling behaviour and movement across nine generational populations which naturally vary in population size. As this study is compares among seasons and avoids the potentially confounding influence of parasitoids it represents a more robust test of the patterns detected by Cade and Cade (1992) and Hissmann (1990).

Methodology

The WildCrickets Field Site and Behavioural Monitoring

The following experiment was conducted at the WildCrickets field site in Asturias, Spain during the 2015 and 2016 reproductive seasons. The study population of field crickets, *G. campestris*, which have been established here for over 40 years (Rolando Rodriguez-Muñoz *pers. comms*), occupy an 800 m^2 area of relatively flat grassland. Crickets create and take refuge in burrows dug into the substrate and it is expected that the vast majority of cricket behaviour takes place in the immediate vicinity of these burrows. Each year, these burrows are detected manually by researchers and identified by a unique number. One of up to 130 video cameras (VivoTek, Taiwan) are deployed at each burrow to record cricket behaviour. The video cameras are deployed prior to crickets emerging as adults and continue recording throughout the reproductive season until the last individual dies. The cameras are equipped with day-night capability and so run continuously throughout this time.

To enable identification of individual crickets, adults are trapped at their burrows, using bespoke traps (see crickettrapping.wordpress.com for details), from two days post-emergence and fitted with a PVC tag. The tag is glued to the pronotum using cyanoacrylate glue and bears a 1-2 character code unique to each individual, which is easily read during video playback. During trapping morphological measurements are also made; specifically individual mass using a precision balance (SNUG II-300, Jadever, Taiwan) and pronotum width. Pronotum width is used as a proximate measure of body size and is measured from top-down photographs of the pronotum

made using a digital camera (PL210, Samsung, South Korea) connected to a dissecting microscope (S6E, Leica Microsystems, Germany). Pronotum width is measured using FIJI image analysis software (Schindelin et al., 2012), and each measurement is calibrated to the character height of the ID tag (see Figure 4). Three photographs of each individual were made, with individual being allowed to slightly adjust its position between photographs, so that an average measurement per individual could be used. This accounts for among-individual error caused by each cricket's position relative to the camera. Following a brief period of recovery, crickets were returned to the burrow from which they were captured. Traps were left in burrows during capture to prevent the burrow being taken over by other individuals.

Data on cricket behaviour was extracted from the recorded videos by manual playback. Playback was conducted in a three-step process: first to detect video sections which contained cricket activity, secondly to extract behavioural data from the video, and finally to check and rectify inconsistencies in the behavioural record. During the second stage, any of 19 key events were recorded (See tables 1 and 2) along with the burrow these events occurred at, the date and time of the event, and the ID's of the crickets involved.

For a full account of the methods highlighted above please refer to Chapter One: General Methodology.

Quantification of male calling song

Male calling effort was estimated through additional video review. The first 10 minutes of each hour that a male cricket was observed by a camera was observed in 1-minute intervals. A binary response was recorded for calling behaviour if the male was observed to call at any time during this 10 minute observation period. This allowed calling effort to be estimated as a proportion of the number of observation periods a male was observed to be singing relative to the number of observation periods for that male. Calling behaviour could be determined from the video recordings due to the raised position of the wings which is distinct to this behaviour and does not coincide with any other activities. A full account of this methodology is given in Chapter One: General Methodology.

Quantification of cricket movement

The data used in these analyses were collected during nine reproductive seasons between 2007 and 2016. Data collected in 2014, are not included as processing was incomplete at the time of writing. Among years, population density varied naturally allowing density-dependent effects on behaviour to be investigated by looking at differences among years. Male movement was estimated using the number of minutes that a male remained at any given burrow as an inverse measure of his movement. This approach is more reliable than estimating absolute movement because cameras are only positioned over known burrows and so when a cricket is not at a burrow his behaviour is unknown and therefore his movements cannot be reliably determined. When a cricket is under a camera however, his behaviour is known exactly and the period of time (recorded in minutes) he spends at that burrow can be accurately determined. The assumption therefore is that males who remain

longer at burrows are those who move among burrows less often. The median of all observed remain times per male was used as a single value estimate of movement for that male. The median was used instead of the mean as the distribution of remain times was highly skewed. The data were filtered such that only males for whom there was a minimum of five observations for remain time were included in the analysis to ensure that the median was a reliable estimate of the number of minutes that individual was expected to spend at any given burrow.

Variation in mate acquisition behaviour among males and among years

To assess the presence of a relationship between LCE and median remain time, a linear model was fitted which included LCE as the dependent variable and median remain time as an independent variable. Population density was fitted as a covariate allowing the effect of population density on LCE to be investigated within the same model.

A positive relationship between LCE and median remain time would indicate that the longer a male spends at a burrow, the more he invests in calling behaviour. This would suggest that males who invest a lot in calling do not move as much as males who invest little, thereby supporting the hypothesis that calling behaviour and movement are potential alternative mating tactics in this system. Conversely, a negative relationship between LCE and median remain time would suggest that males who invest more in calling also move more than males who invest little in calling, therefore suggesting that investment in either behaviour is determined by some condition-dependent mechanism. A negative relationship between LCE and population density would suggest that males called more at low population densities,

as suggested by previous studies, while a positive relationship would suggest that males called more at high population densities. LCE was square-root transformed prior to analysis to achieve a normal distribution and the model fit was assessed from residual diagnostic plots.

An additional linear model was fitted to assess the relationship between median remain time and population density. In this model, median remain time was log-transformed and fitted as the dependent variable. Population density was the only independent variable included. A positive relationship between population density and median remain time would suggest that males moved the most at low population densities while a negative relationship would suggest that males moved most at high population densities.

Survival costs of calling and movement

The potential survival costs of calling and movement behaviours were investigated using two linear mixed models. In each model, lifespan was fitted as the dependent variable while LCE or median remain time were fitted as the only independent variable, respectively. Lifespan is defined here as the total number of days post-emergence that an individual is known to be alive. Year was included as a random effect in both models to control for variation among-years. If moving among burrows imposed a significant increase in the risk of death one would expect lifespan to have a positive relationship with median remain time. Conversely, if LCE is associated with high survival costs, one should expect short lifespans for those individuals who call more often. Therefore, if the relationships between lifespan and LCE, and lifespan and median remain time are in the same direction, it would suggest that one

behaviour imposed a greater survival cost than the other. Individuals investing in the costlier behaviour would need to be able to withstand such a cost and so this result would suggest that poorer quality males may be able to avoid these costs by investing more in the alternative behaviour. However, if these relationships were found to be in opposite directions it would support the hypothesis that both movement and calling effort are determined by individual condition as the longest living crickets would be those who called most often and moved more frequently.

Results

Variation in mating tactics among males

There is a negative relationship between LCE and median remain time (Linear Model: n = 348, $F_{1, 346}$ = 21.3, P < 0.001, Table 20, Figure 13) suggesting that the more a male invests in calling behaviour the more he also moves among burrows. This result supports calling behaviour and movement being determined by a condition-dependent mechanism rather than representing alternative mating tactics.

Table 20: Table summarising the analysis of the effect of median time spent at a burrow and population size on lifetime calling effort. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the minimum adequate model is given providing the model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value.

Maximal model	Lifetime calling effort ~ Median remain time + Population size			
Minimum adequate model	Lifetime calling effort ~ Median remain time + Population size			
Sample size	348			
Model error structure	Square-root transformed			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	F	P
Intercept	0.5	0.0576		
Median remain time	-0.0002	0.00007	21.303	< 0.001
Population size	-0.0008	0.0003	25.284	< 0.001

Variation in mating tactics in response to annual variation in population density

Population density had a negative effect on LCE (Linear Model: $F_{1, 346} = 25.28$, $P < 0.001$, Table 20, Figure 14) indicating that males sing more when the population density is small. There was, however, no significant effect of population density on median remain time (Linear Model: $F_{1, 346} = 1.03$, $P = 0.31$, Table 21).

Table 21: Table summarising the analysis of the effect of population size on the median time spent at a burrow. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as population size had no significant effect on time spent at a burrow. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value for the term of interest are provided.

Maximal model	Median remain time ~ Population size			
Minimum adequate model	Median remain time ~ 1			
Sample size	348			
Model error structure	Log transformed			
Table of Coefficients from maximal model				
	Estimate	95% CI	F	P
Intercept	4.26	0.5048		
Population size	-0.001	0.0027	1.026	0.312

Survival costs of calling and movement

Individuals who lived longer both called more (GLM: n = 462, χ^2= 102.95, d.f. = 1, P < 0.001, Table 22, Figure 15) and spent less time at burrows (GLM: n = 291, χ^2= 19.23, d.f. = 1, P < 0.001, Table 23, Figure 16). This result lends further support to the hypothesis that calling behaviour and movement are condition-dependent traits.

Table 22: Table summarising the analysis of lifetime calling effort on lifespan, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the minimum adequate model is given providing the model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value.

Maximal model	Lifespan ~ Lifetime calling effort + (1|Year)			
Minimum adequate model	Lifespan ~ Lifetime calling effort + (1|Year)			
Sample size	462			
Model error structure	Gaussian			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	X^2	P
Intercept	23.88	3.5535		
Lifetime calling effort	58.47	10.684	102.95	< 0.001

Table 23: Table summarising the analysis of median time spent at a burrow on lifespan, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the minimum adequate model is given providing the model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value.

Maximal model	Lifespan ~ Median remain time + (1|Year)			
Minimum adequate model	Lifespan ~ Median remain time + (1|Year)			
Sample size	291			
Model error structure	Gaussian			
Table of Coefficients from minimum adequate model				
	Estimate	95% CI	X^2 - value	P - value
Intercept	43.32	46.7989		
Median remain time	-0.015	114.5973	19.225	< 0.001

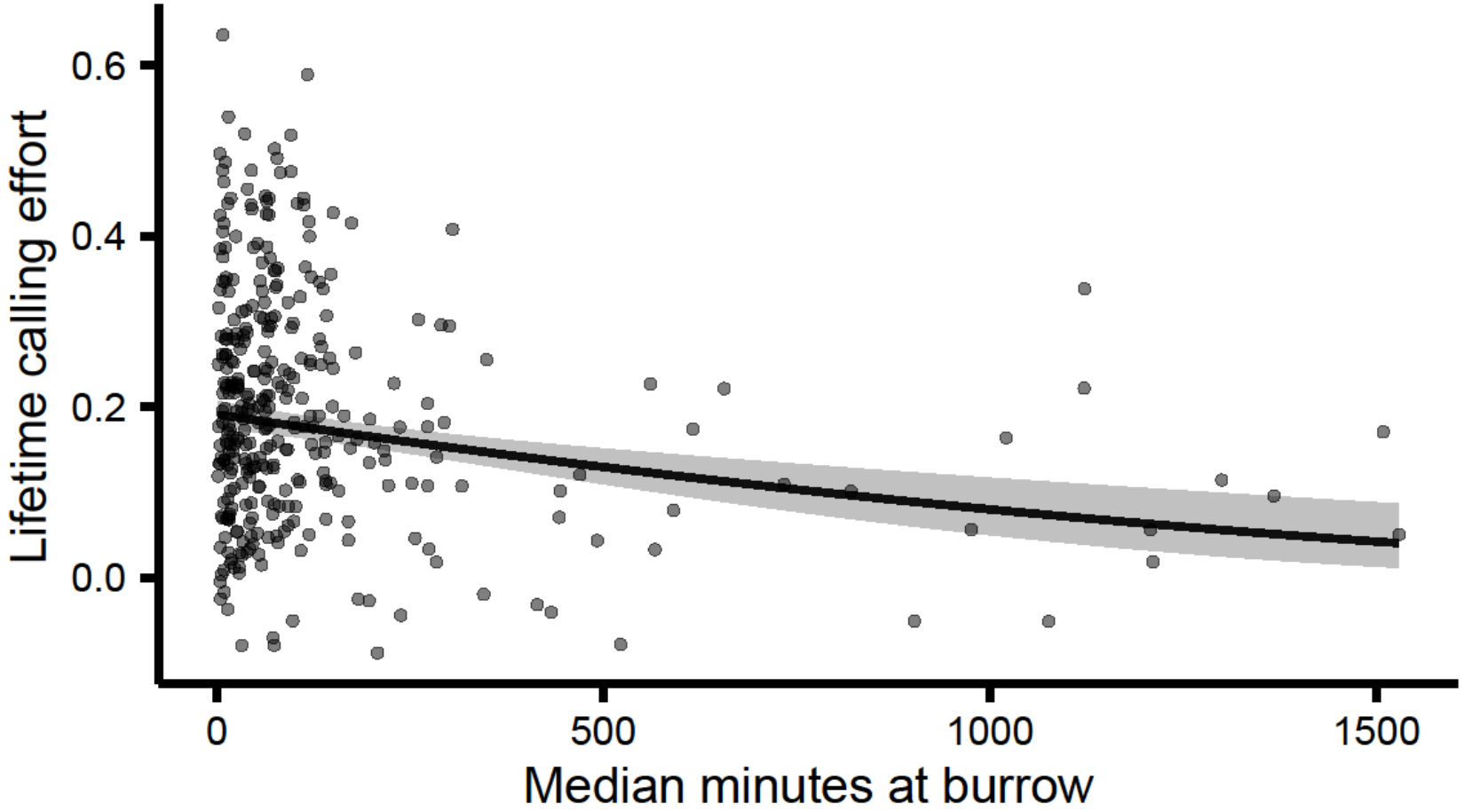

Figure 13: Plot showing the negative relationship between lifetime calling effort and median number of minutes spent at a burrow by each male G. campestris recorded during field seasons between 2007 and 2016 (inclusively). Each point represents an individual male and the line is the prediction of a linear model of LCE in relation to median remain time with population density held at the mean population density. The shaded area represents the 95% confidence interval of this prediction.

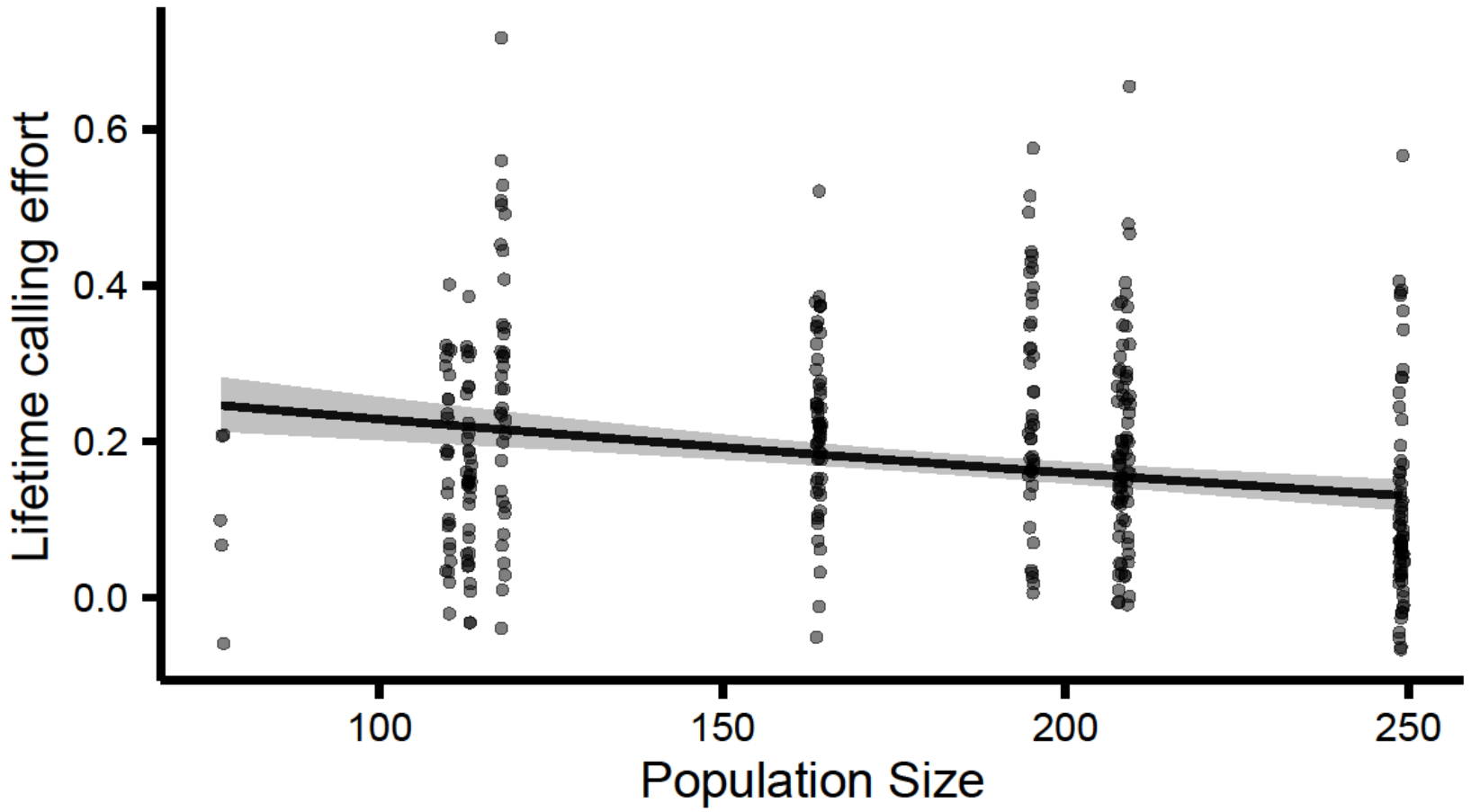

Figure 14: Plot showing the negative relationship between lifetime calling effort and population density for male G. campestris recorded during reproductive seasons between 2007 and 2016. Each point represents an individual male, the line represents the prediction from a linear model of LCE in relation to population density with the median remain time per individual held at the mean. The shaded area represents the 95% confidence interval of this prediction.

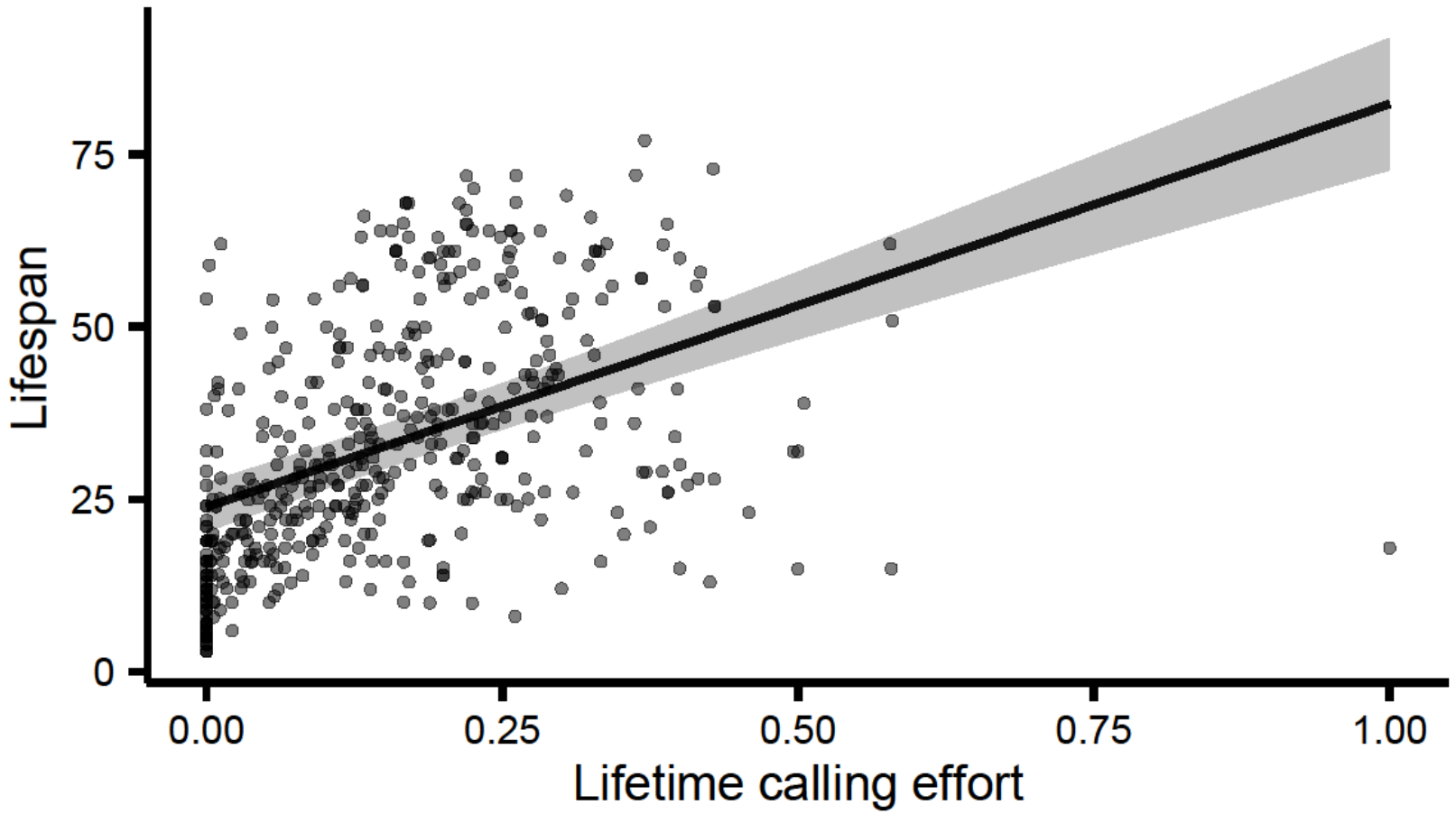

Figure 15: Plot showing the positive relationship between Lifespan and Lifetime calling effort in male G. campestris, recorded during reproductive seasons between 2007 and 2016. Each point represents an individual male while the line is the prediction of a linear model of Lifespan in relation to lifetime calling effort. The shaded area represents the 95% confidence interval of this prediction.

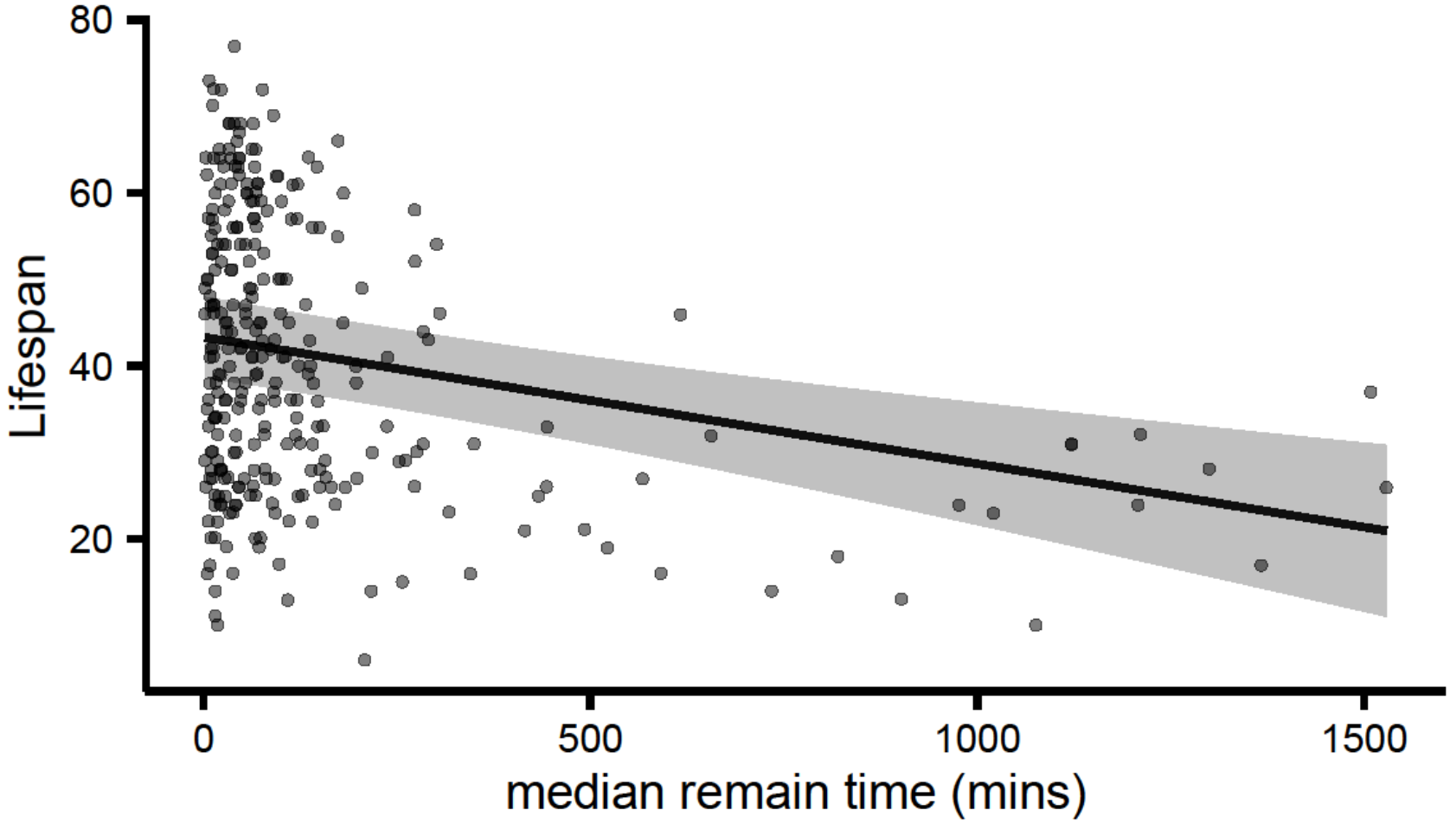

Figure 16: Plot showing the positive relationship between Lifespan and median remain time in male G. campestris, recorded during reproductive seasons between 2007 and 2016. Each point represents an individual male while the line is the prediction of a linear model of Lifespan in relation to median remain time. The shaded area represents the 95% confidence interval of this prediction.

Discussion

Variation in mating tactics among males

This study finds strong evidence against lifetime calling effort and movement behaviour operating as alternative mating tactics. While the lack of a relationship between lifetime calling effort and median remain time would be sufficient to reject the alternative mating tactics hypothesis, the model detects a positive relationship between lifetime calling effort and movement behaviour. This suggests that rather than operating as distinct tactics, lifetime calling effort and movement appear to share a common underlying mechanism. Calling behaviour is well documented as being condition-dependent in crickets (Holzer et al., 2003, Judge et al., 2014, Scheuber et al., 2003, Wagner and Hoback, 1999) and it is plausible that movement should also have some condition-dependent component. The evidence available here, that the mechanism that underlies calling and movement is condition-dependent, is moderate. It is bolstered however, by the finding that both lifetime calling effort and movement have a positive relationship with lifespan. Taken together, these results suggest that rather than poorer condition individuals escaping competition with superior males by adopting a searching strategy, “high quality” crickets both call more and move more than “low quality” crickets.

Variation in mating tactics in response to annual variation in population density

Density dependent effects of mate acquisition tactics have been reported in other insects, specifically the dung fly, *Scathophaga stercoraria* (Stockley and Seal, 2001), and the moth, *Plodia interpunctella* (Gage, 1995), where high

population densities during the larval stage leads to increased investment in testes size in response to perceived sperm competition levels in adulthood. The results reported here suggest that male *G. campestris* invest more in calling effort in response to low population density, similar to patterns found in other populations of field crickets (Cade and Cade, 1992, Hissmann, 1990). As noted previously, these studies offer three possible mechanisms for this pattern of density dependence; the presence of a parasitoid fly, increased costs of defending a burrow associated with population density, and shifts in population density or sex ratio permitting movement as an alternative tactic. These explanations however, are not completely satisfactory.

The presence of the parasitoid fly will almost certainly have some effect on the optimal mating tactic. However, in our population individuals are not known to be at risk from parasitoids, and the main predators are birds and voles which are unlikely to locate prey based on male calling. Hissmann (1990) also detects the same pattern in the absence of such a pressure and so it seems unlikely that this fly is the primary driver of this pattern.

Cade and Cade (1992) conclude that the rising cost of defending a burrow with increasing population density drives behavioural changes in *G. integer*. Again however, the same pattern is found in *G. campestris* who move among burrows very frequently and often vacate them voluntarily, rather than as a result of being evicted. Burrows are a vital commodity for *G. campestris* and so vacating a burrow voluntarily (a behaviour that would appear to be independent of population density) in response to increased competition for burrows would seem counter-intuitive. Furthermore, it is not clear if burrow competition is ever

particularly high in *G. campestris*. Each nymph over-winters in a burrow and while individuals do often usurp burrows from others, they have been observed to enlarge their burrow following moulting (*Pers. obs.)*. We should therefore expect that, due to natural mortality, there will often be more burrows than there are individuals, preventing burrow availability becoming a limiting factor. A male who is evicted from a burrow could therefore move to another available burrow if calling behaviour is the most effective method of mate attraction. However, competition for burrows may exist if there is variation in the benefits that can be gained from occupying certain burrows. Some burrows, for example those closer to female-occupied burrows or situated in denser clusters of burrows, may lead to increased mating success than would more isolated burrows. Studies investigating the cost-benefit structure of defending superior and inferior burrows would be interesting in this context.

Hissmann (1990) conclude that due to the increased chance of encountering a potential mate in high population densities, it becomes viable for males to adopt a silent, searching strategy. However, Hissmann (1990) provides no further explanation as to when and why males should switch strategies, nor why some males continue to sing at high population densities if the optimal strategy is to search.

So, what mechanism is driving the change in calling effort with population density? It is possible that the energetic cost of calling is sufficient to drive the effect. Calling in crickets is a very energetically demanding behaviour (Hack, 1998) therefore if males can avoid the high costs of calling but obtain the same number of females through less demanding searching behaviour they may gain

a net benefit in doing so. As population density declines, the cost of searching increases, and if population density becomes low enough, calling offers the greater pay-off in terms of mating success relative to energy expenditure. Therefore, males should call more at low density populations and less at high density populations. Such a mechanism would be compatible with both Hissmann (1990) and Cade and Cade (1992). One might expect that a reduction in calling effort at high population density, when the number of available females and potential rival males is high, would reduce the strength of sexual selection. Females have been shown to use male calling song to preferentially navigate towards superior males and so a reduction in calling effort may be expected to reduce a female's capacity to assess males, thereby weakening sexual selection. However, this is not expected to have a large effect here as the variation in calling effort, relative to population density is low. Population density most certainly affects the strength of sexual selection, but it appears unlikely that the associated change in calling effort will have a substantial additive effect on the strength of sexual selection.

The above interprets these results from an adaptationist perspective however there are non-adaptive explanations to explain the patterns seen here. For example, it may be that in high density populations, the total amount of movement per unit of space among all individuals is increased relative to low density populations, which can increase encounter rates among individuals, both male and female. Male-male encounters result in fighting behaviour which leads to one individual being forced away from the burrow and so high encounter rates between males may increase the amount of time spent fighting and moving to new burrows when defeated. This would allow males less time to

call at attract females. Similarly, if more females are encountered in high density populations then males may spend less time calling as a result of increased mating rate rather than having made a decision to adopt an alternative tactic. In either case, at lower population density, encounters are less frequent and so singing behaviour is less likely to be interrupted, resulting in greater amounts of singing per individual. Experimental manipulation of population density and encounter rate may be required in order to determine if this is the case.

A second possible explanation for this pattern of calling effort with respect to population density is that an unknown third factor may be influencing both population density and LCE. One example may be variation in the prevailing weather conditions among years. Poor weather conditions may result in low population density and reduced resource availability. Reduced resource availability throughout the meadow may result in lower condition of all males than in more favourable years. With fewer resources males will be less able to withstand the condition-dependent costs of calling and so calling effort is reduced across all males. In years where weather conditions are favourable and resource availability is high, males are less restricted and can invest more in calling effort. There may be some scope to investigate this possibility and other similar explanations (such as the effect of local densities within the meadow) in this system, however with the data available it is unlikely that sufficient power could be achieved to conduct a rigorous test.

An outstanding question that has not been considered here, nor in previous similar studies, is the effect of sex ratio on calling and movement behaviour. While the relationship between calling effort and population density is apparent,

it is not clear what effect the availability of females or the levels of competition with rival males will have. Further studies that investigate the effects of sex ratio on mate acquisition tactics in these insects would be valuable in determining what mechanism underpins the effect of population density on mate acquisition tactics.

Flexible mating tactics in field crickets

This study has found no evidence of calling and movement behaviours acting as alternative, condition-dependent mating tactics in this system. Rather, the results obtained lend support to a single condition-dependent mechanism driving both behaviours. Beyond the individual-level effects of condition, I find some indication that population-level effects may be influencing mating behaviour across the entire male population, which may explain variation in calling effort in relation to population density. It is unclear however if males are making decisions about their investment in calling in response to population density of if the effect is being driven by additional factors such as weather conditions or local population densities. To determine this would require experimental investigation.

Intensive monitoring of natural systems allows far more natural variation to be considered than can be simulated under laboratory conditions. Studying evolutionary processes in the field can reveal the extent of this variation which may have significant implications for our predictions of how populations will respond to changing conditions. Understanding flexibility in mating tactics in response to such conditions is but one example and continued research in this area should be pursued.

Chapter Five

Within- and among-individual variation in female mate choice in wild crickets

Abstract

Female mate choice is a major driver of male sexual traits yet studies of sexual selection often focus on variation in males, neglecting the potentially important effects of within- and among- individual variation in females. Mate choice is costly and females must balance the potential costs of copulation or mate rejection against the benefits of mating. High-quality, good-condition females are expected to withstand greater costs and so individual condition can have a fundamental effect on the choices made. Mating history can also affect mate choice, as the net cost of choice may be higher for virgins than for previously mated females.

To investigate within- and among- individual effects on female mate choice, I used observational data gathered over ten reproductive seasons of a wild population of field crickets. Male crickets defend individual burrows such that most encounters with females do not occur in the presence of other males. Observing natural mating events in the wild therefore approximates the no-choice tests that are frequently used in laboratory studies of mate choice. By observing naturally-occurring mating events, I investigate condition-dependent mate choice by determining the extent to which female mass, size and condition (scaled mass index) predicted how soon after emergence a female begins mating, and mating latency (a measure of female choosiness). I find that neither the time until the first mating event, nor mating latency is predicted by mass, size or condition. These results suggest that the timing of sexual maturity for females in this system is not resource-limited, and that choosiness is not dependent on female condition.

To examine within-individual effects on choosiness, I compared female latency to mate with their second partner relative to their first. I found that significantly more females took longer to mate with their second male than their first, suggesting that previously mated females are choosier than virgins. This result has important implications for studies of mate choice which control for mating history using virgin females.

Introduction

Female mate choice is a major selective pressure driving the evolution of male sexual traits (Darwin, 1871). Until relatively recently, studies of mate choice have focussed on variation in male traits and the effect this has on evolutionary trajectories. The effect of variation in females has received considerably less attention. Variation in mate choice can have a fundamental effect on the strength of sexual selection if biotic or abiotic factors drive females to be more or less discriminating in their decisions (Chaine and Lyon, 2008). Variation in female preferences may even affect the direction of sexual selection. While females are expected to typically prefer dominant males (Qvarnstrom and Forsgren, 1998), in cases where dominant males cause more harm to females during mating than subordinate males (Ophir and Galef, 2003), females in unfavourable conditions may prefer less dominant males so as to avoid such harm; even while dominant males may be the most attractive under favourable conditions. Understanding the effect that variation in female mate choice has on selection is therefore a vital prerequisite to estimating the evolutionary trajectory of a population. Identifying sources of variation in mate choice, both among- and within- individuals, and the consequence of this for mating success is an important issue in sexual selection research.

Female mate choice can be described as the combined outcome of two distinct functions. Preference describes the tendency towards specific traits over others, independent of any costs of choice (Edward, 2014). Choosiness on the other hand is the effort that a female is willing or able to invest in making the choice (Edward, 2014, Jennions and Petrie, 1997). Females who express a preference

for certain male traits may benefit by increasing their probability of mating with higher quality males and thereby securing direct (e.g. nuptial gifts, shared parental care (Kokko, *et al.*, 2003) or indirect fitness benefits (as predicted by good genes or sexy sons' hypotheses (Møller and Alatalo, 1999; Weatherhead and Robertson, 1979)). The magnitude of these benefits depends how variable males are in terms of quality. Exercising mate preferences is, however, expected to come at a cost (Pomiankowski, 1987). Assessment of potential mates can expose females to increased predation or pathogen risk, or be energetically costly and reduce the time available for other activities, such as foraging (Dawkins and Guilford, 1991). Choosiness therefore describes the extent to which a female is willing to suffer these costs to obtain a superior mate. The choosier she is, the more rigidly she sticks to her preference and the greater the costs incurred for ensuring she chooses well. The less choosy she is, the more she is willing to depart from her preference and mate with a broader range of male phenotypes (Jennions and Petrie, 1996).

The relative cost experienced by a female is a trade-off between the cost associated with her preference and the benefit she can gain from mating with a preferred male phenotype. The ability to withstand higher costs of choosiness is expected to vary with respect to female condition in the same way as male ornamentation is expected to vary with condition. Higher condition females, better able to withstand the costs of mate choice, are expected to be choosier, whereas lower quality females, whose net benefit of selecting the most preferred male is lower, are expected to deviate more from their preferences (Cotton, Small and Pomiankowski, 2006). Such variability in the net benefits of mate choice should cause choosiness to be condition (or context), dependent

as the benefit to a female in choosing her mate should be a function of the cost of her choice and her ability to withstand that cost.

Sources of variation in mating decisions

There are numerous environmental and life-history factors that can introduce variation in choosiness, both within and among individuals, as well as at the population level. Among individual variation can arise through differences in the acquisition of resources. High levels of competition for food may lead some individuals to out-compete others. The accumulation of energy stores may allow an individual to better withstand the costs of mate assessment whereas those females with less reserves may settle for less preferred males (Cotton, Small and Pomiankowski, 2006). In times of high resource availability, where competition for resources is lessened, all females may be able to exhibit greater choosiness.

Within-individual variation in choosiness may arise from different priorities at different life stages. The net benefits gained from mating with preferred or less preferred males may change as a female becomes older, and we should not therefore expect to see the same patterns of mate choice in young and old females (Tinghitella, 2014). Similarly, reproductive experience may have an effect on female choosiness as the cost to a virgin female of dying before her next mating opportunity is much higher than for a previously mated female (Kokko and Mappes, 2005).

Population-level variation in choosiness may arise from variation in population densities. When males are plentiful, and mating opportunities are high, females

may be stricter about which males they are willing to mate with than when population density is low. The cost of rejecting a potential mate is much higher when potential mates are scarce (Kokko and Mappes, 2005).

Laboratory studies of variation in mating decisions

In recent years, plasticity in female mate choice has received increased attention and the factors that affect choosiness have been investigated in a number of laboratory studies. Variation in choosiness has been investigated at the among-individual, within-individual, and among-population level, in a number of systems (among-individual: (Brooks and Endler, 2001, Burley and Foster, 2006, Hebets et al., 2008, Kuczynski et al., 2017, Macario et al., 2017), within-individual: (Atwell and Wagner, 2014, Coleman et al., 2004, Kokko and Mappes, 2005), among-population: (Brooks, 2002, Endler and Houde, 1995, Iglesias-Carrasco et al., 2017)).The field cricket, a popular laboratory model system for studies of sexual selection, has been adopted for many such studies, some of which I highlight here.

A typical methodology for investigating among-individual variation is to introduce variation in phenotypic condition by manipulating an individual's diet. This method has been employed to show variation in female mate choice in crickets, and studies consistently find female mate choice to be condition-dependent. In *Teleogryllus commodus*, females raised on a high quality diet express stronger preferences for characters of male calling song, namely frequency and calling rate (Hunt et al., 2005). In *Gryllus pennsylvanicus*, females raised on high- or low-quality diets exhibited similar preferences for male traits, suggesting that preference was independent of individual condition.

Females raised on the low-quality diet were, however, more willing to deviate from their preference and mate with inferior males, suggesting that choosiness can vary with individual condition in this species (Judge et al., 2014). Finally, females of *Oecanthus nigricornis* raised on a high-quality diet expressed stronger preferences for male traits than those raised on a low-quality diet, and were more likely to reject potential mates (Brown, 1997). Variation in individual condition has also been introduced by manipulation of parasite load in a number of evolutionary studies. In the context of female mate choice, it has been shown in *Gryllus lineaticeps* that females infected with a parasite failed to express preferences for any male phenotypes, instead showing equal preference for all phenotypes presented, in contrast to control females (Beckers and Wagner, 2013).

Variation in age and mating experience may introduce within-individual variation in mate choice. A number of studies find age to have a significant effect on female mate choice in crickets. Pacheco et al. (2013) found that age explained 64% of variation in phonotaxis towards male calling song in female *G. lineaticeps,* and that females aged 10 – 13 days post-emergence were the most responsive. Similarly, Gray (1999) found that younger *Acheta domesticus* females were choosier than older females. The effect of variation in age on mate choice is difficult to test as age is often highly correlated with mating experience, which is also reported to affect mate choices (Lickman et al., 1998) which may explain some conflicting results regarding within-individual variation in mate choice. For example, Mautz and Sakaluk (2008) found that older females showed reduced mating latency (a measure for female choosiness) than younger females in *A. domesticus*, but that the number of times a female

had mated previously had no effect on her mating latency. Judge et al. (2010) found the opposite in *G. pennsylvanicus*, reporting that virgin females are more likely to mate, and show reduced mating latency than previously mated females. Furthermore, they also report that age had no effect on the likelihood of mating or on the duration of mating latency in this species. The effect of age on mate choice may also be subject to interactions with other factors. In *G. pennsylvanicus* for example, Atwell and Wagner (2014) report no significant differences in choosiness between females of young, intermediate, and old age. However, young females are shown to exhibit greater plasticity in mate choice than older females, showing greater increases and decreases in choosiness at high and low densities respectively.

Variation in the cost-benefit structure of mate choice is expected to vary with population density as potential mates become more or less likely to be encountered (Cade and Cade, 1992, Hissmann, 1990). Direct evidence of this has been shown in *T. oceanicus* where females raised under simulated high-density conditions showed a reduced willingness to mate than females raised under simulated low-density conditions (Lierheimer and Tinghitella, 2017). In contrast, female *A. domesticus* raised in high and low densities have been found to show no difference in the number of times they initiated courtship with a male. In this study however, females raised in either density treatment approached males to initiate courtship significantly more often than did females raised in isolation (Tinghitella, 2014). This study also reported that older females initiated courtship more often than young females, but found no difference in mating latency associated with age or population density. Beyond the density-dependent costs of mate choice associated with the likelihood of

finding an alternative mate, is the effect that density has on female mate-searching tactics. In a high-density population, one might expect a female to be exposed to greater variation in male quality than in a low-density population and the criteria by which females select males may be sensitive to the levels of variation she is exposed to. For example, in *G. lineaticeps*, females are more likely to reject a male based on song characteristics if she has previously heard a superior song (Wagner et al., 2001), adding further evidence to the hypothesis that females are stricter with their mate choice decisions in larger populations than in small populations.

A final factor that may affect female mate choices is the risk of predation. Any mating activity that increases a female's exposure to predation will be associated with costs relative to the probability of being predated. Predation risk is a dynamic factor that can vary with the time of day or the season, or with a range of environmental factors. When predation risk is high, the potential cost to a female is high, as being predated severely reduces her fitness (perhaps to zero if early in life). It may be expected then that a female's motivation to mate, or the mate choice criteria she uses, will vary according to perceived predation risk. Some evidence of this has been provided in crickets where female *Gryllus rubens* that emerge in the autumn, a time when they are vulnerable to a fatality-inducing parasitoid fly that locates crickets by male calling song, are less attracted to male calling song than females collected in spring, who are not subjected to parasitism by the fly (Velez and Brockmann, 2006). This result suggests that population level variation in female choice may be mediated by fluctuation in predation risk. Furthermore, *G. lineaticeps* females have been found to be less choosy if they have been exposed to predator cues compared

to control females (Atwell and Wagner, 2015). This may be due to the increase in costs associated with rejecting a potential mate if the probability of being predated before the next potential mate is encountered is high.

Investigating female preference in the wild

The studies highlighted above have been useful in exploring the effects that female variation might have on sexual selection. Each have, however, been conducted under laboratory conditions. While a very effective tool, laboratory studies are limited in the extent to which they reliably predict the natural world. By design, such studies remove vast amounts of natural variation which, when restored, may change expectations of studied traits. As a result, laboratory experiments investigating mate choice risk drawing unreliable conclusions.

It is important then to gain insights into the extent to which the findings of laboratory studies hold true in the wild, where the full complement of natural and environment variation is restored. Increasing attention is being given to behavioural differences of organisms in the laboratory versus the wild. To fully understand the factors that influence female mate choice, and the effect that this will have on sexual selection, effort must be made to conduct rigorous studies in as natural an environment as possible.

The WildCrickets camera network allows intensive monitoring of a population of wild field crickets, *Gryllus campestris*, by deploying video cameras that can capture behavioural events across an individual's entire adult lifespan in the individual's natural environment. This allows naturally varying population densities and resource availability, and preserves free movement of individuals

as well as the risk of predation. Using this system, I firstly investigate if females in good condition begin to mate sooner after emergence than those in poorer condition. Secondly, I investigate how aspects of individual condition affect female choosiness by measuring mating latencies. If females in greater condition exhibit longer mating latency this would provide support to the findings of laboratory studies from a study conducted in natural environment of my study system. On the other hand, should condition be found not to influence mating latency then this would indicate that the laboratory environment may influence mating behaviour, leading to misestimation of female mate choice in nature. Finally, I investigate how female reproductive experience affects choosiness in the wild. As laboratory studies often use virgins to control for potential effects of mating experience it is important to understand how female mate choice decisions might change with subsequent matings before broad conclusions of the effect of female choice can be made.

Methodology

1st mating

The effect of two physical traits, and a combination of these traits, on the age at which a female begins mating were investigated These were; pronotum width, used as a proximate measure of body size (see **Chapter One** for details), body mass at emergence (collected as described in **Chapter One**), and condition index. Condition index was estimated using the scaled mass index method described in Peig & Green (2009). This method has been shown to provide a more reliable estimate of condition than previous methods (Peig and Green, 2009). The method is described by the following formula:

$$Condition = M\left(\frac{L_0}{L}\right)^{b_{SMA}}$$

Where M is the mass of the individual, L is the body measurement which in this case is pronotum width, L_0 is the mean pronotum width for females in this population, and the scaling component, b_{SMA}, is derived from standardised major axis regression of the log of M on the log of L.

To allow comparison among years, and to avoid potentially confounding factors caused by earlier- or later-emerging females, adult age when first mated was used as the response variable in these analyses. Adult age at first mating is calculated as the number of days between emergence until the female's first mating event, inclusively. Only females whose emergence date could be reliably estimated to within one day were included in these analyses.

An obvious variable which may affect the mating behaviour of females is ambient temperature; both at the time of mating and, more importantly, during maturation. Ideally, the mean temperature between emergence and first mating would be included in these models to account for variation in mating behaviour attributed to variation in metabolic rate, as a result of variation in temperature. However, temperature in this system is confounded with age, as the ambient temperature naturally increases throughout the reproductive season. The result is that females who, for whatever reason, begin mating at a later age, include higher temperatures in their mean temperature estimate. This creates an apparent positive relationship between age at first mating and mean temperature which is due to a statistical artefact rather than a biological mechanism. Including the temperature during the first mating would have the same effect as females whose first mating occurs when they are older are more likely to be mating when the temperature is warmer. With these data it is not possible to determine if this is due to higher temperatures encouraging mating, or if the preferred age at which to begin mating occurs later in the year.

Monitoring effort was included in the models to account for variation in the reliability of female age estimates due to variation in monitoring intensity. This value was the total amount of time that a female was observed on a video camera between emergence and her first observed mating event (recording in minutes).

The data used in these analyses were collect during ten reproductive seasons between 2006 and 2016. Data were collected during the 2014 reproductive season but are not included in these analyses as processing was incomplete at

the time of writing. The distribution of female age at the time of first mating was not normal, being zero-bounded and right-skewed. Log-transformation of the response variable was sufficient to provide a suitable fit for a gaussian linear model. High levels of correlation between the three traits of interest, body size, mass at emergence and condition, prevented them being included in a single model. Therefore, three separate models were constructed, each containing monitoring effort and one of the traits of interest as independent variables. In each model, year was included as a random factor to account for any variation in mating behaviour among years.

Female mating latency

Mating latency is estimated here as the time interval between a male and female meeting at the burrow entrance until the female mounts the male to commence mating. Mating latency has been recorded to the resolution of one minute in ten reproductive seasons between 2006 and 2016 (excluding 2014). In 63% of recorded mating events, mating commenced within the first five minutes of the female meeting the male. In the remaining 47% of mating evenings, mating occurred between 6 minutes and 43 hours after meeting. While investigation of the effect of female condition or life history on mating latency would be most robustly conducted by modelling latency as a continuous function of the condition or life-history trait in question under the framework of a generalised linear model, none of the classic error structures produced an acceptable model fit with this data. For this reason, mating latency was categorised as fast mating (mating occurred within five minutes of meeting), and slow mating (mating latency exceeded five minutes). The probability of a fast mating occurring is then modelled as a function of condition or life-history trait.

Latency as a function of female identity

To establish if there was any repeatability in mating latency within females, mating latency was modelled as a function of female ID. The response variable was the proportion of fast mating latencies for each female relative to the number of slow latencies for each female. Female ID was included as the only fixed effect. The relationship was modelled using a GLMM with a binomial error structure and year fitted as a random factor to account for any variation among years. As described in a similar analysis in **Chapter Two**, a null result for female ID would indicate that fast and slow matings were distributed randomly among females, suggesting little repeatability in mating latency within females. A significant effect of female ID however, would suggest high repeatability in mating latency within females, indicating that some females tend to mate inherently faster than others.

Among individual variation in latency

Once repeatable variation in mating latency among females was determined, I attempted to explain such variation in relation to measurable aspects of female condition; body size, mass at emergence and condition. As above, high correlation between these variables prevented them from being included in the same model, and so three separate GLMs were constructed. Each model included the trait of interest and year as fixed effects, and the proportion of fast relative to slow mating latencies as the response variable. Year was included as a covariate to determine if among-year effects influenced the probability of fast or slow mating events occurring.

Within individual variation in latency

To determine if there was any within-female variance in mating latency that could be explained by variation in life history, the change in latency between a female's first mating with her first male, and her first mating with her second male was investigated. A significant change in mating latency would be indicative of within-individual variation in response to mating status (virgin versus mated). The difference in latency was calculated by subtracting the mating latency with the second male from the mating latency of the first male:

$$\Delta Latency = Latency_1 - Latency_2$$

Where $Latency_1$ is the latency to mate with the first male, and $Latency_2$ is the latency to mate with the second male. As such, negative values represent an increase in latency when mating with the second male. The distribution of latency differences was highly kurtotic with a large peak near zero and long, shallow tails in both directions. As such, the data were analysed by a non-parametric binomial test. The total number of negative changes in latency totalled 199, while the total number of positive changes in latency totalled 144, leaving 56 cases where female latency remained the same at the first and second mating. As these cases where mating latency did not change were not informative as to the probability that a female would become choosier, they were discarded. The binomial test was performed to determine if the change in latency between the first and second male was negative more often than expected, indicating an increase in female choosiness for mated females relative to virgin females.

Results

None of the physical traits investigated (condition index, body size or mass at emergence) had a significant effect on the age at which a female first mated (GLMM: condition index: n = 218, χ^2 = 0.56, d.f. = 1, P = 0.45, Table 24; pronotum width: mean ± SE = 7.51 ± 0.024 mm, n = 218, χ^2 = 0.01, d.f. = 1, P = 0.9, Table 25; mass at emergence: mean ± SE = 0.96 ± 0.008 g, n = 230, χ^2 = 0.25, d.f. = 1, P = 0.62, Table 26). Pre-mating monitoring effort had a significant effect on the observed age at which a female first mated (GLMM: n = 218, χ^2 = 6.58, d.f. = 1, P = 0.01, n = 230, χ^2 = 6.35, d.f. = 1, P = 0.01) and was included in all final models.

Mating events occurring within or exceeding five minutes of a male and female meeting were found to be non-randomly distributed among females (GLM: χ^2 = 821.93, d.f. = 550, P < 0.001). This indicates that there is some aspect of females which affects how soon after meeting she will mate with a male that is independent of any aspect of that male. However, this is not due to variation in condition index (GLM: n = 258, χ^2 = 0.1, d.f. = 1, P = 0.75, Table 27), pronotum width (GLM: n = 261, χ^2 = 0.11, d.f. = 1, P = 0.74, Table 28), or the mass at emergence (GLM: n = 276, χ^2 = 0.43, d.f. = 1, P = 0.51, Table 29) of the female. The year of study had no effect on the likelihood that a mating event would occur within five minutes of meeting (GLM: n = 276, χ^2 = 5.07, d.f. = 8, P = 0.75).

Within individual variation in mating latency was detected in relation to mating history. The proportion of females who took longer to mate with their second

male than their first male was 0.58, a significant departure from the expected value of 0.5 (Binomial test: n = 343, P = 0.003, Figure 17), suggesting that mated females are choosier than virgin females.

Table 24: Table summarising the analysis of condition index and monitoring effort on the age at which a female first mates, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as monitoring effort is not biologically informative but is included to control for variation in observation intensity. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value are given.

Maximal model	Age at first mating ~ Monitoring effort + Condition index value + (1|Year)			
Minimum adequate model	Age at first mating ~ Monitoring effort + (1|Year)			
Sample size	218			
Model error structure	Log transformed			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	2.195	0.5313		
Monitoring effort	0.02	0.0126	6.887	0.008
Condition index value	0.21	0.5318	0.558	0.455

Table 25: Table summarising the analysis of body size and monitoring effort on the age at which a female first mates, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as monitoring effort is not biologically informative but is included to control for variation in observation intensity. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value are given.

Maximal model	Age at first mating ~ Monitoring effort + Body size + (1|Year)			
Minimum adequate model	Age at first mating ~ Monitoring effort + (1|Year)			
Sample size	218			
Model error structure	Log transformed			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	2.45	1.2373		
Monitoring effort	0.016	0.0126	6.579	0.01
Body size	-0.008	0.1646	0.014	0.91

Table 26: Table summarising the analysis of mass at emergence and monitoring effort on the age at which a female first mates, with year fitted as a random factor. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given as monitoring effort is not biologically informative but is included to control for variation in observation intensity. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value are given.

Maximal model	Age at first mating ~ Monitoring effort + Mass at emergence + (1|Year)			
Minimum adequate model	Age at first mating ~ Monitoring effort + (1|Year)			
Sample size	230			
Model error structure	Log transformed			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	2.27	0.4962		
Monitoring effort	0.016	0.0124	6.389	0.011
Mass at emergence	0.14	0.4995	0.246	0.62

Table 27: Table summarising the analysis of condition index and year on mating latency. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given no independent variable had a significant effect on mating latency. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value are given.

Maximal model	Proportion of short mating latencies relative to long mating latencies ~ Condition index value + Year			
Minimum adequate model	Proportion of short mating latencies relative to long mating latencies ~ 1			
Sample size	258			
Model error structure	Binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	0.38	0.8006		
Condition index	0.13	0.8025	0.102	0.7489
Year (2008)	0.15	0.5026	5.934	0.655
Year (2009)	-0.05	0.311	5.934	0.655
Year (2010)	-0.05	0.4244	5.934	0.655
Year (2011)	-0.21	0.3585	5.934	0.655
Year (2012)	-0.03	0.7202	5.934	0.655
Year (2013)	0.11	0.3243	5.934	0.655
Year (2015)	0.05	0.4235	5.934	0.655
Year (2016)	0.4	0.6356	5.934	0.655

Table 28: Table summarising the analysis of body size and year on mating latency. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given no independent variable had a significant effect on mating latency. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value are given.

Maximal model	Proportion of short mating latencies relative to long mating latencies ~ Female Body size + Year			
Minimum adequate model	Proportion of short mating latencies relative to long mating latencies ~ 1			
Sample size	261			
Model error structure	Binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2	P
Intercept	0.76	1.8893		
Female body size	-0.041	0.2446	0.108	0.743
Year (2008)	0.1	0.4939	5.682	0.683
Year (2009)	-0.04	0.3054	5.682	0.683
Year (2010)	-0.05	0.4247	5.682	0.683
Year (2011)	-0.22	0.3672	5.682	0.683
Year (2012)	-0.05	0.7212	5.682	0.683
Year (2013)	0.09	0.3306	5.682	0.683
Year (2015)	0.04	0.4195	5.682	0.683
Year (2016)	0.39	0.6358	5.682	0.683

Table 29: Table summarising the analysis of mass at emergence and year on mating latency. The table gives the formula of the maximal and minimum adequate model obtained following model simplification. The sample size and the specified error structure of the model are given. The table of coefficients from the maximal model is given no independent variable had a significant effect on mating latency. The model estimate and 95% confidence interval, the test statistic obtained when models including and excluding the term of interest were compared, and the P value are given.

Maximal model	Proportion of short mating latencies relative to long mating latencies ~ Mass at emergence + Year			
Minimum adequate model	Proportion of short mating latencies relative to long mating latencies ~ 1			
Sample size	276			
Model error structure	Binomial			
Table of Coefficients from maximal model				
	Estimate	95% CI	X^2 - value	P - value
Intercept	0.14	0.8095		
Mass at emergence	0.26	0.7772	0.102	0.749
Year (2008)	0.21	0.502	5.934	0.655
Year (2009)	0.008	0.2953	5.934	0.655
Year (2010)	0.05	0.4134	5.934	0.655
Year (2011)	-0.102	0.3541	5.934	0.655
Year (2012)	0.016	0.6662	5.934	0.655
Year (2013)	0.17	0.3306	5.934	0.655
Year (2015)	0.09	0.4156	5.934	0.655
Year (2016)	0.46	0.6347	5.934	0.655

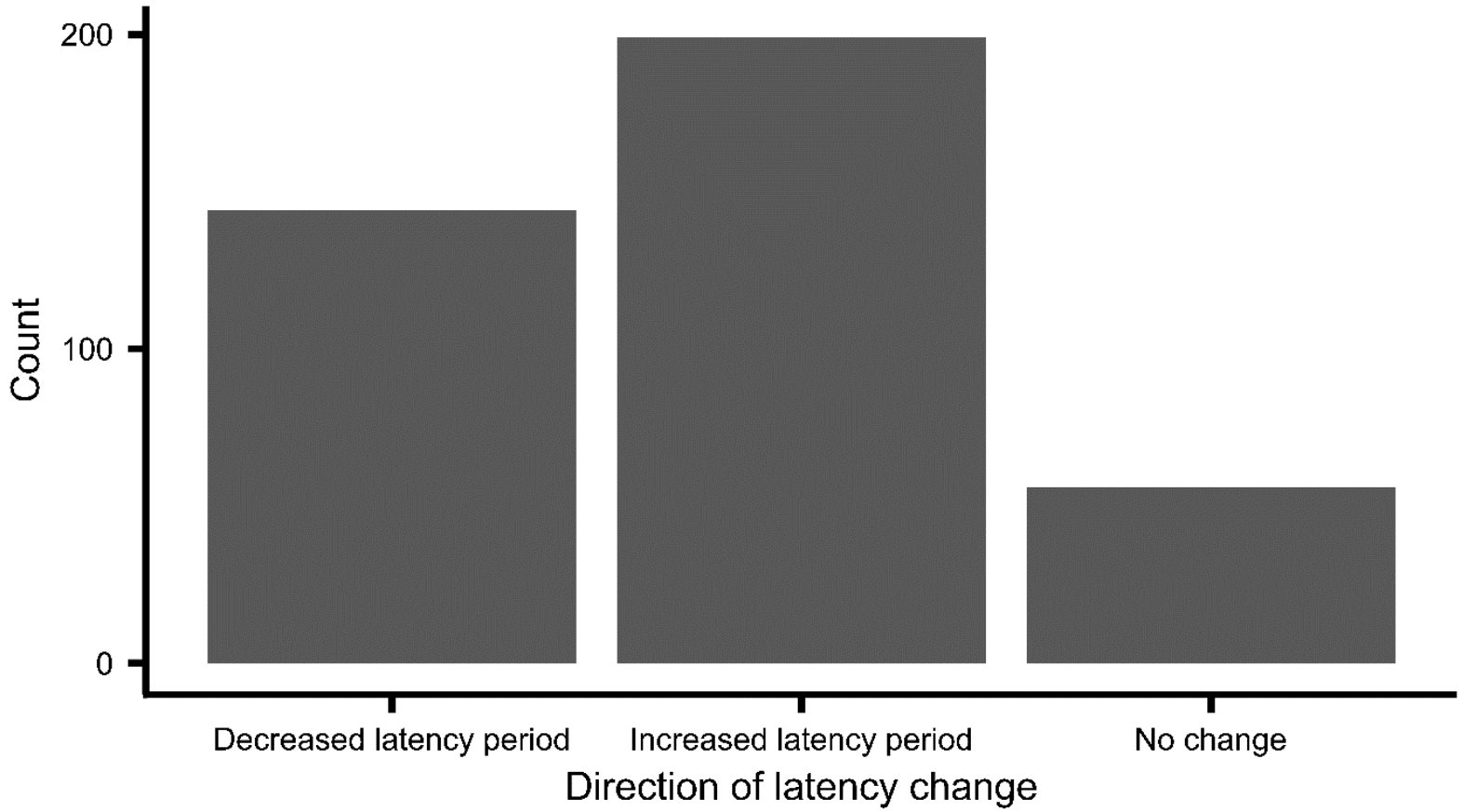

Figure 17: Plot showing the number of females who mated sooner with their second male than their first (decreased latency), the number of females who took longer to mate with their second male than their first (increased latency), and those females who did not detectably change their mating latency between the first and second male. The difference in the number of females who increased and decreased their latencies is significant (P = 0.003).

Discussion

The aim of my study was to determine if the same variation in mate choice decisions that are often reported in laboratory studies are observed in a wild population. I investigated the same levels of effects (among-individual, within-individual and population level) as are typical of laboratory studies to examine the possibility that laboratory studies overestimate the biological significance of observed effects.

Among-individual variation in mate choice

Laboratory studies consistently report that variation in mate choice is condition-dependent (see Cotton et al., 2006). Laboratory studies often employ a no-choice mating design and measure the latency to commence mating following the meeting of a male and female (Cotton et al., 2006a, Immonen et al., 2009, Lindström and Lehtonen, 2013, Moore and Moore, 2001, Schultzhaus et al., 2017, Shackleton et al., 2005). I employed a similar approach but failed to detect a significant effect of either individual condition (as estimated by condition index), body mass, or body size on variation in mating latency.

The marked differences in the conditions under which laboratory and wild animals live may contribute to the failure to replicate the same patterns of condition dependent mate choice in the wild as are seen in the laboratory. Laboratory artefacts such as fixed resource availability, unrealistic population densities, and a lack of predation may contribute to the discrepancy in results here.

A large proportion of condition-dependent phenotypic variation is expected to be a product of the resources that an individual can acquire from the environment and subsequently allocated to development and maintenance (Rowe and Houle, 1996). Often, in laboratory studies, food is provided *ad libitum* and so variation introduced by foraging efficiency is eroded. Alternatively, introducing variation in dietary treatments can be used as a way of creating condition-dependent variation among individuals, however this is typically restricted to two groups (high-quality and low-quality). The removal of natural variation may affect mate choice in two ways. When food is provided *ad libitum* the result may be that there is little condition-dependent variation in males for females to assess, or all females are in sufficient condition to be very choosy. Conversely, if individuals are separated into treatment groups, the difference between the two groups can be artificially exaggerated such that there is an unnaturally large difference between “high condition” males and “low condition” males, and so female mate choices may be overestimated.

Further as it is not often practical to maintain natural population densities in the laboratory, and even less so to maintain natural fluctuation in population density, studies often raise experimental animals in arbitrarily defined densities, same-sex groups, or in isolation. This can influence how individuals perceive competition and sex-ratio which can have a marked effect on mate choice (Jirotkul, 1999b; Lierheimer and Tinghitella, 2017; Tinghitella, 2014). Many studies then offer naïve females a choice of males or male stimuli in dichotomous or no-choice designs ((Cotton et al., 2006a, Immonen et al., 2009, Lindström and Lehtonen, 2013, Moore and Moore, 2001, Schultzhaus et al., 2017, Shackleton et al., 2005)) meaning that a female’s interaction with an

experimental male is often her first interaction with any male mimicking an extremely low population density with low mate availability. As mate availability decreases, the risk of dying before encountering another potential mate increases (Kokko and Mappes, 2005) and so we might expect female choosiness to be generally low as an artefact of such experimental designs. Nevertheless, laboratory-based, no-choice experiments are a powerful design so it is possible that the effects they detect are real and most operate in nature. Failure to detect these effects in the present study may therefore be the result of increased noise in the wild system relative to the laboratory, which is sufficient to mask this effect. The results reported here should not then be taken as outright evidence against condition-dependent variation in mate choice but rather as an indication that condition is not a dominant factor in structuring such variation.

I also investigated condition dependence in the age at which females begin mating. Females who are able to begin mating earlier in life may avoid potentially increased costs of mating late in life (i.e. it may be less costly to mate while young than mate while old). The ability to mate early in life will be dependent on the speed at which a female develops, which is dependent at least in part on her developmental environment and her ability to exploit that environment. As such, a female's condition at sexual maturity may have important fitness consequences and affect selection. While my study has observed substantial variation in the age at which females begin mating, this cannot be explained by variation in condition, body size, or body mass, independently; but this is not to say that age is not an important factor in mate choice. Age may be very relevant later in life and could provide a major source

of within-individual variation in mate choice, but this has not been investigated here.

Within-individual variation in mate choice

Theory predicts that females should be less choosy in selecting their first mate than when selecting subsequent mates. The so called “wallflower effect” predicts that because the cost of dying before mating is greater than the cost of mating with an inferior male, it does not benefit virgin females to be choosy (Kokko and Mappes, 2005). The present study has detected the wallflower effect in a wild system finding significantly more females mate with their first male sooner after meeting than they do with their second male. While the noise in this natural system relative to a laboratory system may have prevented the detection of an effect of condition on mating latency, there is not sufficient noise to mask the effect of mating status. This suggests that the effect of mating status on choosiness is rather substantial, or is at least greater than the effect of condition. Similar results have been found in guppies, *Poecilia reticulata*, where females were found to be relatively less responsive to the second male they encountered than the first (Pitcher *et al*. 2003), and in newts, *Titurus vulgaris*, where singly-mated females were found to be more willing to mate with a second male when that male was superior to her previous mate (Gabor and Halliday, 1997). Changes in mate choice associated with mating experience may not be linear, and so virgin females may not represent a random sample of a population, as all mating events contribute to the gene pool of the next generation. Mate choice decisions of virgin females may not, therefore, be representative of the “average” mate choice decisions made in a population – or even for each individual. Despite this, a using virgins remains a common

method of controlling for variation in mating status. Future laboratory studies should therefore be mindful of the limitation this places on results obtained from experiments using virgin females. Such results may be relevant only to virgins and may underestimate the effects of the traits being investigated. Where possible, using females in a range of mating statuses would be valuable.

Among-population level variation in mate choice

The present study uses non-overlapping generations of the study population to investigate among-population level variation in mate choice, but does not detect an effect of year on mating latency. This indicates that choosiness is consistent with respect to year and that factors fluctuating among years, such as resource abundance, weather conditions and population density, do not have a significant effect on choosiness. However, only 10 generations are available (providing just 10 data points) which may not provide a sufficient sample size with which to detect such effects. Furthermore, variation among generations may be a rather weak proxy for among-population variation as generations of a single population are more likely to be living in similar conditions than populations that are truly distinct. It remains important then to consider the potential effect that environmental variation may have on mate choice among populations. Future wild studies investigating such effects would be welcomed.

Considerations for future work

Whilst my study has not detected an effect of condition on mate choice, limitations in the study mean that there may still be large effects of condition on mate choice that have gone undetected. Condition is a dynamic trait that is variable throughout the life of an individual but practical limitations restricted me

to consider condition only at the time of emergence. The lack of condition-dependent variation in choosiness reported here should not therefore be taken outright as evidence against condition-dependent, among-individual variation in mate choice in the wild. Further rigorous studies that investigate changes in mate choice with changes in condition throughout life in a wild system are required, and those that directly manipulate individual condition would be particularly valuable.

An active area of evolution and ecology research at present is the effect that ageing and senescence has on life history and behavioural traits. Mating behaviour is almost certainly affected by age and age-related decline in condition, and so significant within-individual variation in mate choice should be expected with respect to age. At different life stages, the priority of a female towards growth and development, reproduction, and somatic maintenance will vary, and this may have a substantial effect on choosiness. A female whose priorities favour reproduction may be far less choosy than she would be when her priorities favour somatic maintenance, for example. Similarly, a female at the end of her life may be willing to mate with any male if she expects to die soon anyway (Kodric-Brown and Nicoletto, 2000). Studying the effects of ageing is a challenge, and disentangling baseline mortality and age-related mortality risk is a difficult task. Furthermore, age is often confounded with mating experience, as multiply mated females are likely to be older than virgin females. Separating the effects of age and mating experience on mate choice is particularly challenging in the wild, but warrants investigation and could yield fascinating results.

My study reveals an effect of mating status on mate choice in the wild, however, I am unable to determine the precise nature of this relationship. It is not clear from these results if the change in mating latency detected between mating with the first and second male is a step change that happens only at this juncture, marking the change in status from virgin to mated female. Mating latency with subsequent males may remain similar to that of the second male as the female remains “mated”. Alternatively, the relationship between mating latency and mating history may be curved, with female choosiness increasing with each subsequent mating as the relative fitness benefits of mating declines. Differences in choosiness may then be expected, not just between virgin and mated females, but also between “first mated”, “second mated, and “third mated” (etc.) females. Determining the shape of this relationship would be very interesting, however, attempts to do so will suffer from the confounding effects of age highlighted above, which must first be considered.

Chapter Six

General Discussion

Summary of findings

I began this project with the aim of implementing an experimental manipulation of condition in a wild population of field crickets to examine the effect this had on sexually selected traits. I hypothesised that individuals provided with a dietary supplement would be in better condition and therefore achieve greater mating success than control males, thereby inferring an alignment of natural and sexual selection. Due to difficulties encountered in the execution of this experiment, the approach was only possible in one of the chapters presented here, and so I also explored a number of aspects of condition-dependent sexual selection using an observational dataset compiled over 10 years.

In Chapter Two I presented a direct test of sexual selection for condition-dependent traits. I found that dietary supplementation caused an increase in mass at emergence, indicating that supplemented crickets were consuming the food source provided. It was not possible however to determine conclusively from the available data if this increase in mass was associated with allocation to tissues or if a large portion of food was stored in the gut and later excreted. A positive effect of dietary supplementation on body size, which would require allocation of additional resources, was not detected. This does not necessarily mean that additional resources were not invested in development as the incremental growth pattern of field crickets limited the potential for overall growth in this experiment. In both years, the experiment began shortly before the crickets were expected to reach adulthood and so most crickets only moulted a few times following supplementation. The effect of dietary supplementation on body size would therefore have to have been rather

extreme for such an effect to be detected. Nonetheless, the likelihood that a male would be observed calling increased with the number of days the food supplement was provided. This does suggest that calling effort is resource limited and that the food supplement was utilised by fed individuals.

Despite a small increase in calling effort with respect to dietary supplementation, food-supplemented males did not achieve greater mating success. Without differential mating success, it is unlikely that dietary supplementation would lead to differential reproductive success, unless there was an unobserved effect of dietary supplementation in post-copulatory competition. As such, dietary supplementation is unlikely to have influenced sexual selection and so an alignment between natural and sexual selection cannot be inferred here.

It should be noted that I did not attempt to control for the effect of dietary supplementation in subsequent chapters, despite the 2015 and 2016 field seasons being included in those analyses. Controlling for potential effects of diet was considered but determined not to be important here. The only variables found to be significantly affected by supplementation, and included in subsequent chapters, were calling effort, which affects the 16 males supplemented as adults in 2016, and body mass, which was a component of female condition used in Chapter Five, which had no significant effect on any measured variable. Two possible solutions to potential issues of including supplemented individuals were considered, but not implemented. Firstly, dietary supplementation could be accounted for in subsequent models by including the number of days fed as a covariate. This would, however, result in the majority of

individuals having zero values for this variable, which is statistically problematic. An alternative option would be to discard all supplemented individuals from subsequent analyses. This too is undesirable as these individuals do provide useful information and their dietary status is not expected to introduce bias that would lead to Type 1 errors in subsequent analyses. I therefore considered the inclusion of these individuals to be the most conservative approach, and one likely to increase the power of subsequent analyses.

Chapter Three considered the role of overall body size in determining mating success in *G. campestris*. Surprisingly, my results suggest that body size is not a useful predictor of mating success, in this species, in their natural environment. This is in contrast to previous findings from a number of laboratory studies in crickets that do report body size to influence mating success (Bateman, 1998a, Bateman et al., 2001, Champagnon and del Castillo, 2008, Fedorka and Mousseau, 2002, Forrest et al., 1991, Gray, 1997, Ponce-Wainer and del Castillo, 2008, Ritz and Kohler, 2010, Saleh et al., 2014, Shackleton et al., 2005, Simmons, 1995). While the results presented here do not nullify these studies, they highlight the value of studying natural systems. It is important to acknowledge that within the considerable environmental noise associated with the natural world, variables such as body size, which almost certainly have some effect, are far more difficult to detect. This should prompt us to consider their biological significance, as certainly in this system, body size does not appear to be as important in determining mating success as was expected.

While body size does not appear to have a meaningful effect on mating success, the relative size of individuals in a fight does predict the outcome of

that fight. As the loser of a fight is most frequently evicted from the burrow, there are obvious costs to defeat, including increased predation risk and exposure to adverse environmental conditions. The magnitude of such costs, and therefore their biological significance, are not easy to measure. It is unclear if female field crickets consider fighting ability in their mate choice decisions (Bretman et al., 2006, Brown, 2008, Loranger and Bertram, 2016, Nelson and Nolen, 1997, Savage et al., 2004, Shackleton et al., 2005), however exclusion from a burrow could have a significant, indirect effect on male mating opportunities. For example, exclusion from a socially important burrow, one that sits within a cluster of female-occupied burrows perhaps, could significantly decrease a male's mating opportunities. Further investigation of the relationships between aggressive behaviour, fighting success, survival and reproduction in field crickets would be valuable. My results, however, do not explain variation in mating success and so, while body size can be easily considered a condition-dependent trait it is not likely to be under sexual selection.

In Chapter Four I searched for evidence of a trade-off between calling effort and movement of males which could facilitate a condition-dependent switch-point in mate-searching strategies. Rather than detecting such a trade-off, I instead found a positive relationship between calling and movement. This provides strong evidence against these behaviours acting as alternative mate-searching tactics, effectively nullifying the hypothesis that switches between calling and searching tactics are mediated by individual condition. I did however find evidence to support previous studies of a density-dependent, flexible mate-searching system operating in this species. At greater population densities

males called less, presumably encountering mates when moving among burrows, whereas males invested more in calling behaviour in lower density populations. Determining the precise mechanisms which lead to such an effect is difficult as population density is likely to be determined by the environmental conditions prior to the reproductive season, which are not accurately known here and are unlikely to have been measured in previous similar studies. It is unclear if this effect may be driven by density *per se* or as a result of other environmental conditions which also happen to affect density.

As calling behaviour is known to be condition-dependent, one might expect sexual selection to be stronger in low density populations as females can use the calling signal to better discriminate among potential mates. However, males who invested more in calling effort, and so were presumably in better condition, also moved among burrows more. As such, these individuals could still be expected to be more successful in higher density populations, despite the decline in calling, due to an increase in mate encounter rate.

Chapter Five considered the effect of female condition in driving sexual selection in this system. I found no evidence that either the age at which a female begins mating, nor how choosy a female will be when presented with a potential mate, can be predicted by the condition, determined using a scaled mass index approach (Peig and Green, 2009), of that female. This is surprising as costs of mating have been reported for females of many taxa and therefore the relative costs and benefits of choosiness are expected to vary with condition in a similar manner to the expression of sexually selected traits in males. Female choosiness is, however, influenced by previous mating history.

Significantly more females increased their latency to mate when mating with their second male relative to their first male, than decreased their latency or remained unchanged. This has important implications for future studies of mate choice as the effect of mating history is often controlled by using virgin females. Such studies may be inadvertently underestimating the effect of the traits they are investigating as a virgin female's choosiness may not be indicative of her choosiness later in life. As females in many systems, certainly in field crickets, will mate more times as a "previously mated female" than as a "virgin female", measuring only the choosiness of virgins may significantly skew the interpretation of any detected effects. Indeed, it is not known if this change in choosiness is detected only between the first and second mating events, or if changes in choosiness can be observed with each successive mating, or if the magnitude of such changes vary. Researchers should be cautious of this, and try to include females with a range of mating histories in their designs, or pre-determine the effects of mating history in their specific system.

Implications for sexual selection theory

Taken together, the work of this thesis does not provide strong evidence of condition-dependent sexual selection occurring in nature. I do identify some condition-dependent traits but find that these do not cause significant variation in mating success in this system. If mating success is not biased towards males in better condition, then sexual selection is unable to act on those traits. As such, these traits are unable to provide an alignment between natural and sexual selection as hypothesised. That being said, due to practical limitations and technical issues encountered in the production of this thesis, the evidence gathered here is not sufficient to refute the operation of condition-dependent sexual selection in this system. Conducting studies such as this in a natural system introduces a substantial amount of noise in the dataset, for example, behaviour can be affected by weather conditions (*G. campestris* activity is reduced in windy or rainy conditions, *pers. obs.*) or temperature variation (Kindle et al., 2006, Martin et al., 2000, Walker and Cade, 2003). As such, large variances in the measured traits (coupled with small samples sizes available for the experiment conducted in Chapter One) may have obscured biological effects which have been detected in the laboratory. Despite this noise however, not all effects are obscured. Some effects have been large enough to be detected here such as, for example, the effects of dietary supplementation on calling effort, relative body size on fight outcome and mating status on female choosiness. This suggests that if effects that I have failed to detect do exist, but are obscured by noise, the size of these effects must be smaller than those effects that I did detect. This would infer then that, as another example, the effect of diet on calling is greater than the effect of diet on mating success, suggesting that there must be other factors involved in mating success and

calling effort is not the only determinant, even though no effect of condition is detected.

Two further technical factors may have diminished my ability to detect important biological effects; a short feeding time in the experiment conducted in chapter two, and poor measures of condition in other chapters. Dietary manipulation was found to cause an increase in mass at emergence and calling effort in male crickets in Chapter Two, however this was not sufficient to influence mating success – suggesting a weak or absent biological effect of supplementation on calling effort. In order for a significant difference in mating success to be detected with respect to supplementation, the supplement would have to increase calling effort (or any other sexually selected trait) enough to be detectable above the considerable natural variation in male calling effort. It is possible that with continued provisioning the cumulative effect of dietary supplementation would eventually have a large enough effect on calling effort so as to produce a biologically significant change in mating success. In nature, variation in foraging ability or metabolic efficiency can be genetically determined (Rowe and Houle, 1996), in which case variation in resource acquisition is present among males from hatching. Therefore, small effects could accumulate over a long period of time and lead to the expected differences in calling behaviour and mating success associated with condition. Even with a high value food supplement, the short period over which crickets were fed here may not have allowed biological effects to become detectable. Detecting such an effect without experimental manipulation would be very difficult. It is also possible that the effect of supplementation is short-lived and therefore lost shortly after supplementation ceases. As the data used in these analyses were

drawn from the entire reproductive season, which continues for several weeks after the supplementation phase of the experiment, supplemented males may have returned to the control state, making it harder to detect an effect of supplementation on calling effort. Investigating the effect of supplementation on calling effort during and after the feeding experiment might highlight the timeframe over which the effect is prominent and whether or not effects do indeed accumulate. However, calling is known to be weather dependent and varies with ambient temperature (Ciceran, Murray and Rowell, 1994; Martin, Gray and Cade, 1999). This introduces considerable environmental noise which, coupled with the fact that only 16 males were available for investigation, makes it unlikely that this data would have sufficient power with which to investigate this question thoroughly.

In chapter Five, condition at the time of emergence is estimated from body size, which is fixed at adulthood, and mass, which is continuously variable. Due to issues encountered in recapturing individuals, I was unable to keep a reliable record of individual mass throughout the reproductive seasons. As such, the condition metric used in these analyses does not necessarily represent the condition of the individual at the precise moment of mating, but is instead the best available estimate. Studies that precisely track fluctuation in body mass of wild crickets across the reproductive season would be invaluable as mass is likely to influence many other behaviours. To my knowledge, reliable estimates of the rate of change in body mass, and the within- and among-individual variation in body mass, have not been produced for any insect under natural conditions. In Chapter Four, condition is not considered directly but instead the presence of a trade-off that would indicate a condition-dependent mechanism

was investigated, based upon assumptions of the system. No such trade-off is detected but it is not clear if these assumptions were appropriate and condition-dependence is not a factor here, or if the assumptions were incorrect.

The huge amount of environmental noise in this data makes detecting effects very difficult and so it is not possible to make firm yes or no conclusions. However, incorporating this environmental noise in to the study is one of the major motivations of this work. Biological processes are operating amongst this noise and so the true biological significance of any trait is dependent upon this noise. If the effect size of any trait considered in this thesis is not sufficient to be detected by the analyses implemented, then it is important to consider how biologically significant they are with respect to sexual selection.

Implications for the design of wild studies

This thesis highlights a need for field studies that can provide insights in to the relative biological importance, under natural conditions, of effects which have been discovered in the laboratory. I find that increased expression of a condition-dependent trait alone does not necessarily produce a strong enough effect to influence male mating success given the many other factors involved in the wild. I find that greater body size can help a male win a fight but that this still may not mean that he is reproductively superior. My results also warn that controlling for mating status by using virgin females may be misleading. It is therefore of great importance that studies of sexual selection consider how laboratory-based results may manifest under natural conditions and indeed the most powerful approach to addressing these issues is to increasingly conduct studies in the field, or conduct laboratory and field studies in tandem. With rapid development in technology there is tremendous potential to begin observing and conducting relatively non-invasive experiments on wild systems. It is certainly not possible to implement such approaches for all systems and all questions, but where there is the possibility of such an approach, it should be pursued with vigour. While practical challenges have left the present work somewhat open-ended, future similar studies are vital to clarifying those elements of uncertainty discussed here. While it was initially intended that all chapters in this thesis would include an experimental component, a number of practical challenges encountered with regards to dietary supplementation has meant that much of the work has been observation-based, and where the experiment was successfully employed, the sample sizes available have been low. Uncovering these challenges has played a large role in the production of this thesis, but solving them will help improve the quality of execution for such

designs and therefore improve our ability to determine the actions of natural processes, in natural conditions. There is great potential for future studies that might adopt a similar kind of approach and so I here highlight some important points for consideration by researchers who intend to employ such designs.

Balancing observation and restriction of movement

A major difficulty in executing the experiment was maintaining awareness of cricket locations so that food could be deployed accurately each day. This will undoubtedly pose a challenge to any such study as one must balance the need to control the system with the desire to preserve natural behaviour. Dietary supplementation could be administered to the intended recipient without error if recipients were constrained to a specified area, as done by Holzer et al. (2003), however this interferes with the natural movement of the individual and interactions between individuals. My approach was to monitor tagged crickets using the WildCrickets camera network, redistributing food according to cricket movements as detected through daily video monitoring. This preserves natural movement of individuals but is far more time and labour intensive, and limited the duration of the experiment as cricket movement increases throughout the reproductive season, eventually becoming unmanageable. Difficulty in maintaining a perfect feeding regime has forced dietary supplementation to be considered a continuous variable here, rather than a categorical variable as initially intended. Future researchers should consider the likelihood of an imperfect deployment and consider how best to analyse their data should discrete categories be unavailable.

Food source and supplementation efficacy

Diet quality, specifically the macronutrient content of the supplement, plays a key role in insect life history and so should be given particular consideration in the design of manipulation experiments (Bunning et al., 2016). Furthermore sex-specific effects of different macronutrient ratios have been detected in insects and should be considered with respect to any traits targeted by the experiment in question (Rapkin et al., 2017, Rapkin et al., 2018). Furthermore, the value of a given food resource may vary with age or among life stages (Barbosa et al., 1986, Milanović et al., 2016).

The food source must therefore be carefully determined as the application of an arbitrary additional resource may have little effect if it does not contain a beneficial macronutrient content. A more specific and complex feeding regime does however lead to more complicated logistics and will likely make correct delivery of the supplement more challenging. In many cases, one might expect a simple, balanced and highly nutritious supplement to be the most efficient, but this requires the investigator to determine what this supplement should be composed of, and devise reliable ways to measure the efficacy of the supplement.

Duration and timing of supplementation

I found that the effect of dietary supplementation may not be large and so to maximise variation in condition, supplementation should be applied over as long a period as possible and at a time that maximises investment in resource allocation. Supplementation may have different effects on different traits at different life stages, and so this should be considered in the design of the study.

When and for how long supplementation can be maintained may also influence considerations of food source (matching dietary components to life stages), observation methods (temporal differences in behaviour) and indeed the study system itself.

Suitability of study system

Practical considerations

The effects of dietary supplementation may be small and so large sample sizes may be required, with individuals being intensively monitored. This constrains the suitability of species to such studies, not just in terms of the behaviours of the animals but the practicalities associated with monitoring in the natural environment of that species. The burrow-keeping habits of *G. campestris* have made this species particularly amenable to this type of study as the majority of activity is conducted at easily detected sites. Other species with defined homes, such as nesting birds may also be appropriate for such studies, while more transient species or those with large home ranges may not be. Similarly, populations that live in habitats that are easily accessed are far more appropriate to this type of study than those who live in, for example, dense vegetation.

Ethical considerations

Investigators should consider the effect that the experiment will have on the next generation of their system. The goal of dietary manipulation is to alter the pattern of selection in the population. The application of dietary supplements may lead to the increased reproductive success of individuals bearing disadvantageous genotypes, thereby promoting the spread of “bad” genes

rather than “good”. While over a few generations this may not have a drastic effect, one should consider possible ramifications. Such considerations may constrain the species or populations that would be appropriate for these studies. While understanding the role of sexual selection in adaptation might be most useful for conservation efforts of threatened species, direct manipulation of a threatened species may not be appropriate.

Concluding Remarks

Experimental and laboratory studies have been, and continue to be, an extremely useful tool in the study of sexual selection. Nevertheless, the usefulness of investigating the biological significance of effects detected under laboratory conditions has been highlighted here, as well as in other studies of entire wild populations such as the St. Kilda Soay Sheep Project (http://soaysheep.biology.ed.ac.uk), the Isle of Rum Red Deer Project (http://rumdeer.biology.ed.ac.uk), the Wytham Great Tits (http://wythamtits.com), the Kalahari Meerkat Project (http://kalahari-meerkats.com/kmp), and the Banded Mongoose Project (http://socialisresearch.org/about-the-banded-mongoose-project). If our goal as biologists is to understand the natural world as clearly as possible, then development of methods which allow the investigation of natural systems, but maintain as many of the benefits of laboratory studies as possible, will be very valuable. Such wild studies are becoming more feasible and are revealing the potential complementarity between laboratory and natural systems. Future work which combines the control of laboratory experiments with the context of natural systems, by carrying out studies in parallel, will be a very powerful approach with which to continue tackling many challenging questions in evolutionary biology; not least the effects of condition-dependent sexual selection.

Appendices

Appendix 1: Review of papers investigating alignment of natural and sexual selection

Summary of studies which investigate alignment between natural and sexual selection highlighting how, where applicable, variation in fitness and the opportunity for sexual selection have been introduced or amplified, the measurements that the conclusions have been based on (fitness comparison), and whether or not support for an alignment between natural and sexual selection has been found.

	Manipulation				
Study System	**Sexual Selection**	**Fitness**	**Fitness comparison**	**Support**	**Citation**
Baker's yeast (*Saccharomyces cerevisiae*)	Manipulation of sex ratios	Novel environment	Ratio of Malthusian parameters	X	Reding et al. (2013)
Fruit fly *(Drosophila melanogaster)*	Monogamy versus polyandry	Thermal stress	Net reproductive rate	X	Holland (2002)
		Chemical mutagenesis	Net reproductive rate, fecundity and viability	X	Hollis and Houle (2011)
		Local adaptation	Frequency of deleterious homozygotes	✓	Hollis et al. (2009)
		None	Ability to purge deleterious alleles	X	Arbuthnott and Rundle (2012)

	Sexually signalling versus non-signalling populations	Thermal stress	Rate of adaptation to thermal stress	X	Cabral and Holland (2014)
	Common garden competition	Local adaptation	Competitive ability of adapted versus maladapted males	X	Arbuthnott and Rundle (2014)
	Variation in required mate searching effort	Mutant versus wildtype males	Reproductive success	✓	MacLellan et al. (2009)
	None	Thermal stress	Competitive ability of adapted versus maladapted males	✓	Dolgin et al. (2006)
		Local adaptation	Offspring fitness in well-adapted versus maladapted populations	✓*	Long et al. (2012)
Fruit fly (*Drosophila serrata)*	Monogamy versus polyandry	Detection of receptive female	Reproductive success	✓	Hollis and Kawecki (2014)
		None	Net reproductive rate	X	Holland and Rice (1999)
			Correlation of body size and survival	✓	Promislow et al. (1998)

			Offspring survival	✓	Partridge (1980)
	Factorial suppression of natural and sexual selection	Novel environment	Changes in allele frequency	X	Chenoweth et al. (2015)
			Population fitness	X	Rundle et al. (2006)
Fruit fly (Drosophila subobscura)	Monogamy versus polyandry	Radiomutagenesis	Mutation load	✓	Savic Veselinovic et al. (2013)
	None		Mating behaviour and mating success of irradiated versus control males	✓	Veselinovic et al. (2017)
Fruit fly hybrid (Drosophila serrata x Drosophila birchii)	Factorial suppression of natural and sexual selection	Hybridisation	Promotion of mate recognition	✓	Blows (2002)
Yellow Dung fly (*Scathophaga stercoraria*)	Monogamy versus polyandry	None	Longevity and lifetime reproductive success of females	X	Martin et al. (2004)
Flour beetle (*Tribolium castaneum*)	Monogamy versus polyandry	Variable diet quality	Reproductive success of females	✓	Grazer et al. (2014)

		Pesticide exposure	Adaptation to novel pesticide	✓	Jacomb et al. (2016)
		None	Mutation load	✓	Lumley et al. (2015)
Seed beetle (*Callosobruchas maculatus*)	Monogamy versus polyandry	Novel environment	Rate of adaptation to a novel host	✓*	Fricke and Arnqvist (2007)
		Radiomutagenesis	Egg-to-adult survivorship and number of adult progeny from females	X	Power and Holman (2015)
	None		Rate of mutation purging in males versus females	✓	Grieshop et al. (2016)
Horned dung beetle (*Onthophagus taurus)*	Monogamy versus polyandry	Radiomutagenesis	Rate of mutation purging	✓	Almbro and Simmons (2014)
House cricket (*Acheta domesticus*)	None	None	Correlation of sexually selected components of calling song and haemocyte load	✓	Ryder and Siva-Jothy (2000)
Jamaican field cricket (*Gryllus assimilis)*	None	None	Offspring viability from matings with preferred versus non-preferred mates	X	Bertram et al. (2016)

Field cricket (*Gryllus bimaculatus*)	None	None	Reproductive success of females who chose their mates versus those allocated a mate	✓	Simmons (1987)
Field cricket (*Gryllus campestris*)	None	Dietary supplementation	Attractiveness of males with increased resources versus a natural control	✓	(Holzer et al., 2003)
Bulb mite (*Rhyzoglyphus robini*)	Monogamy versus polyandry	Inbreeding depression	Extinction rate	✓	Jarzebowska and Radwan (2010)
		Radiomutagenesis	Rate of purging of deleterious alleles	X	Plesnar et al. (2011)
			F2 fitness following F0 radiomutagenesis	✓	Radwan (2004)
	Sexually selected populations versus non-selected populations	Absence of selection	Fitness between sexually selected and no selection populations	X	Radwan et al. (2004)
Guppy (*Poecilia reticulata*)	None	None	Correlation between male attractiveness and survival	X	Brooks (2000)

			Male attractiveness and offspring fitness for males of various sizes	✓	Reynolds and Gross (1992)
Mexican spadefoot toad tadpoles (*Spea multiplicata*)	Females mated to preferred or rejected males	Resource competition	Adaptation to resource competition	X	Pfennig et al. (2015)
Gray tree frog (*Hyla versicolor*)	None	None	Performance of offspring of males with high- or low-quality calls	✓	Welch et al. (1998)
Poison dart frogs *(Dendrobates leucomelas* & *Epipedobates tricolor)*	None	None	Offspring quality of males with high versus low quality calls	✓	Forsman and Hagman (2006)
House mice (*Mus musculus*)	Females mated to preferred or rejected males	None	Viability of offspring	✓	Drickamer et al. (2000)
		Inoculation with *Salmonella sp.*	Ability of offspring to survive infection	✓	Raveh et al. (2014)
House mice (*Mus domesticus*)	Monogamy versus polyandry	None	Embryo viability	✓	Firman and Simmons (2012)
Bushy-tailed woodrat (*Neotoma cinerea*)	None	None	Female preference for males across a range of	✓	Weber et al. (2007)

			values for condition dependent traits		
Collared flycatcher (*Ficedula albicollis*)	None	None	Variation in nestling condition with respect to a male secondary sexual character	✓	Sheldon et al. (1997)
Peacock (*Pavo cristatus*)	Forced random mating with male of known status	None	Growth and survival of offspring	✓	Petrie (1994)

* The study finds evidence for an alignment between natural and sexual selection but only when the population is maladapted or just beginning to adapt. When the population is well adapted, sexual selection is found to have a negative effect on fitness.

Appendix 2a: Review of papers investigating sexual selection in Gryllid crickets

Summary of sexual selection studies which use Gryllid crickets; showing the species, study format, a summary of the major analyses, and a summary of the conclusions. An initial literature list was compiled by querying the Web of Science database using one of three search terms; “sexual selection”, “mating success” and “mate choice”. The initial list was filtered to remove less relevant studies, such as those related to speciation, sexual conflict, or physiological mechanisms. Other relevant papers known to me have been included however this table is not intended as a comprehensive review. Formats are coded as: LE = laboratory experiment, LO = laboratory observation, FE = field experiment, FO = field observation. Studies highlighted in green find mating success or a sexual trait to be influenced by body mass or body size. Those highlighted in orange find mating success or sexual traits to be independent of body mass or body size. A bibliography for the cited references appears in appendix 2b.

Species	Format	Analysis Structure			Conclusion	Ref.
Acheta domesticus	LE	Response to female chemical cues	~	Mating Status + Diet (cross-factored treatments)	Male mating status influenced how long a male would spend in contact with a chemical cue, but the likelihood that a male would interact with the cue at all was independent of his mating status or diet.	1
	LO	Calling effort Call characteristics	~	Morphology Biochemical compound concentrations	Calling was influenced by morphology and a single tested biochemical compound.	2

	LE	Calling effort (principal component)	~	Diet	Investment in calling increased with dietary phosphorous concentration.	3
	LE	Survival Reproductive output Nymph quality	~	Choice treatment (cryptic choice vs no-choice)	The ability to remove a spermatophore had no effect on female survival, reproductive output, or nymph quality.	4
	LO	Female preference (calling song)	~	Body size $_{M}$	Females preferred the calling song of larger males, determined by the difference in the number of pulses per chirp between large and small males.	5
	LE	Calling Song (Attractive vs Unattractive)	~	Moult mass + Age + Diet + Trial time + Trial mass + Reproductive investment index	Only female age and trial time influenced the probability that a female would choose the attractive male's calling song.	6
	LE	Mating Latency Spermatophore retention time	~	Age $_{M}$ + Mating status (cross-factored treatments)	Latency to mate was influenced by age, but neither age nor mating status affected spermatophore retention time.	7

	LO	Likelihood of mating	~	Fighting ability	There was no difference in courtship behaviour between winning and losing males, but females were more likely to mate with winning males.	8
	LO	Mating Latency	~	Latency to chirp Latency to court	Latency to mate was independent of male courtship latency.	8
	LE	Incorporation of sperm in spermatophore Female sperm storage	~	Sperm age	Younger sperm were more likely to be incorporated into the spermatophore and more likely to be stored by females than older sperm.	9
	LO	Calling song (Syllables per chirp)	~	Haemocyte load + Capsule volume + Body size $_{M}$	Larger males produced more intense (louder) calls. Haemocyte load and male body size had a positive effect on the number of syllables per chirp.	10
	LE	Male attractiveness	~	Fighting ability	Winning males were more likely to be mounted by females than loosing males.	11

	LE	Female preference (calling song) Likelihood of Mounting Mounting latency	~ Body size $_M$ Body mass $_M$	Females preferred the calls of large males. Heavier males were more likely to be mounted and were mounted sooner than lighter males.	12
Allonemobious socius	LE	Number of eggs laid Time spent in second mount Spermatophore retention time Spermatophore transfer success	~ Nuptial gift availability	Females who received the nuptial gift laid more eggs and remained in the second mount position longer. Spermatophore retention time was greater when the nuptial gift was provided but post-copulatory retention time did not differ between treatments and spermatophore transfer success was equal between groups.	13
	LO	Spermatophore Attachment (Successful vs Unsuccessful)	~ Body size $_M$ Spermatophore mass	Successful males were significantly larger than unsuccessful males, but there was no difference in spermatophore mass.	14

	LO	Spermatophore mass	~	Body size $_M$	Spermatophore mass was independent of body size.	14
	LE	Calling song (Various components) Nuptial gift size Mating success Courtship duration	~	Immune activation + Femur length	Immune-system activated males produced shorter inter-pulse intervals than control males and provide smaller nuptial gifts. Mating success and courtship duration were unaffected by immune activation.	15
	LE	Immune function	~	Mating success	Haemocyte load, lytic activity and encapsulation ability declined with increased mating effort. Increased female mating effort was associated with an increase in phenoloxidase activity.	16
	LE	Female preference	~	Calling song (chirp rate, chirp duration of stimulus)	Female preference was influenced by chirp duration but not chirp rate.	17
	LO	Mating success	~	Body size $_F$ + Body size $_M$ +	Mating success was independent of body size.	18

Gryllodes sigillatus	LO	Mate guarding period Spermatophore retention time	~	Guarding (male removed vs undisturbed) + Rival (present vs absent) + Additional female (present vs absent)	Females retained spermatophores longest if guarding males were undisturbed and when rivals were absent. Mate presence had a stronger effect on spermatophore retention time than rival presence. Mate guarding time was reduced when additional females were introduced.	19
	LO	Female preference	~	Calling song (Various components) + Body size $_M$	Females preferred large males with short song pulses.	20
	LE	Spermatophore Retention Time	~	Mate familiarity (novel vs familiar) + Inbreeding status (inbred vs outbred)	Females removed familiar, inbred male's spermatophores faster than novel inbred males, but familiarity had no effect with outbred males.	21
	LE	Mating Latency Spermatophore retention Time	~	Attractiveness $_M$ (sequential cross-factored treatments)	Latency to mate and spermatophore retention time was affected by the first male's attractiveness, but not the second's	22

	LO	Male mating success	~	Courtship song (rate) + Harp size + Body Mass + Condition	Courtship call rate had a significant effect on male mating success, with the most successful males having the highest calling rates.	23
	LO	Calling effort	~	Body size $_M$ + Endurance + Lytic activity + Resting metabolic rate	Calling effort was affected by body size and endurance, but not by lytic activity and resting metabolic rate.	24
	LE	Mounting success	~	Cercectomization status	Cercectomized males were less likely to be mounted by females.	25
Gryllodes sigillatus, Gryllus veletis, Gryllus texensis	LE	Sperm allocation	~	Competition treatment (No rival vs 1 rival vs 6 rivals)	The addition of a single rival increased sperm allocation in male *G. veletis*, but further additional rivals decreased sperm allocation. In *G. texensis* there was no density-dependent effect of rivals on sperm allocation, whereas in *G. sigilatus* sperm allocation dependence on the order in which the males experienced the different rival treatments.	26

Gryllodes supplicans	LE	Spermatophore retention time	~	Guarding treatment $_{M}$ (undisturbed vs removed vs removed and replaced)	There was no significant difference in spermatophore retention time among treatments.	27
	LE	Spermatophore retention time Number of mounts of rival male	~	Guarding treatment $_{M}$ (male guard vs male removed)	There was no significant difference in spermatophore retention time among treatments.	27
Gryllus assimilis	LO	Fecundity Egg viability Juvenile viability Productivity	~	Mating treatment (preferred vs non-preferred male) + Body size $_{M}$ + Body size $_{F}$	Females preferred larger, heavier males expressing more elaborate signals but mating treatment had no effect on female fecundity or offspring viability. Larger females laid more eggs but morphology had no effect on offspring viability.	28
	LE	Number of eggs laid Number of viable offspring	~	Female attractiveness (preferred vs non-preferred)	The number of eggs produced and offspring viability were independent of female attractiveness.	29
	LE	Female preference	~	Fighting ability	Female preferences were not affected by male fight outcome.	30

	LE	Likelihood of mating	~	Male preference status (preferred vs non-preferred)	Mating was more likely to occur when a female was paired with her preferred male. Females were more likely to mount a larger, fight-winning male when given a choice.	30
	LE	Female preference	~	Calling song (Sound pressure level)	Females preferred call playbacks with higher sound pressure levels.	31
	LE	Female mounting frequency	~	Muted or intact male	Muted males were less successful in eliciting female mounting.	32
	LE	Calling song (Principal Components)	~	Diet + Life stage at application (Cross-factored treatments High- or low-quality diet to juvenile or Adult crickets)	The interaction between diet and life stage of application had a significant effect on male call characteristics, with individuals fed the poor diet then the good diet expressing reduced chirp rates and durations while males fed the good diet followed by the poor diet had lower call carrier frequencies.	33
Gryllus bimaculatus	LE	Likelihood of mating	~	Male familiarity (novel versus familiar)	Females preferred novel males over familiar males.	34

	LE	Male courtship effort	~	Body size $_F$	Investment in courtship was independent of female size for virgin males, but mated males preferentially courted large females.	35
	LE	Mating Latency Spermatophore retention time Number of rejections	~	Body size M (sequential cross-factored treatments)	Smaller males were more likely to be rejected, although first-mated males had an advantage in spermatophore retention time.	36
	LE	Number of eggs laid (daily)	~	Dominance rank $_M$ + Body size $_M$ + Body size $_F$	Females laid more eggs when mated to dominant, but not necessarily larger, males.	37
	LE	Oviposition Female Preference Longevity	~	Treatment with seminal protein vs control	Male seminal proteins had no effect on female preference or oviposition, and females treated with seminal proteins died sooner than control females.	38
	LE	Female preference	~	Encapsulation rate + Lytic activity + Body mass $_M$	Females preferred males who had greater encapsulation rates. Lytic activity and body mass had no effect on female preference.	39

	LE	Reproductive success	~	Choice (male size) vs No choice	The ability to choose a large male had a significant effect on oviposition rate and the number of eggs produced, but not the percentage of eggs that hatched.	40
	FO	Paired status (Paired vs Solitary)	~	Morphology $_{M}$ + Calling + Age + Infection status	Parasite prevalence did not influence pairing success or calling behaviour. Infected, solitary males were younger than infected paired males, while overall, older males were more likely to be paired.	41
	LE	Number of eggs laid Proportion of eggs laid Proportion of eggs hatched	~	Remating treatment (Single male vs Two males vs Four males)	The number and proportion of eggs laid was independent of treatment group, but the proportion of eggs that hatched was greatest for females who had mated with multiple males.	42
	LO	Calling song (Carrier frequency)	~	Body size $_{M}$	No relationship between body size and call characteristics.	43
	LE	Calling effort	~	Age $_{M}$	Males invested less energy in calling with age.	44
	LE	Female preference	~	Calling song (young male vs old male)	Significantly more females preferred the young-type call than the old-type call.	44

	LE	Mean lifetime calling activity per male	~	Body size $_M$	Larger males called more often.	44
	LE	Mating success $_M$	~	Mating success (Father)	The offspring of males who were successful in a dichotomous mating tournament were more likely to also be successful in a dichotomous mating tournament themselves.	45
	LE	Spermatophore Retention Time Fate of spermatophore Latency to mate with rival	~	Guard treatment (Present vs Absent) + Rival treatment (Present vs Absent)) + Female mating status	Spermatophore retention time was greater for virgin females in all treatments. There was a significant interaction between rival presence and guard presence on spermatophore attachment time. The presence of a guarding male significantly reduced the latency to mate with the rival and the number of times the spermatophore was dislodged by rival copulation attempts.	46

	LE	Female preference (Calling song - "young male" vs "old male")	~	Age $_F$ Song amplitude	Females preferred the call of the younger male when amplitudes were standardised, independent of female age. If the old male call was loudest however, no general preference was detected.	47
Gryllus campestris	FE	Probability of calling Number of females attracted	~	Diet	Diet-supplement males were more likely to be observed calling and attracted more females than control males.	48
	LE	Calling song (Daily calling rate) Wing morphology Body condition Body size (Male)	~	Diet + Immune challenge	Body condition, size, and mass were positively affected by diet, but unaffected by immune challenge, while wing morphology was affected by both diet and immune challenge. Calling rate was unaffected by diet or immune challenge.	49
	FO	Mating Success	~	Body size $_M$ + Body condition	Larger males, in better condition, obtained more mates.	50
	FO	Paired status (Paired vs Solitary)	~	Body size $_M$ + Age + Tibia asymmetry	Larger, older, and more symmetrical males were more likely to be found paired.	51

Gryllus firmus	LO	Reproductive success	~	Body size $_{M}$	Larger males sired a greater proportion of offspring.	52
Gryllus integer	FO	Calling duration Individual movement	~	Population density	Males called more and moved shorter distances at low-density populations than high-density populations.	53
	LE	Female preference	~	Pheromones $_{M}$ (Dominant vs Subordinate vs Control) Mating status $_{M}$	Females preferred the pheromone samples of dominant males than subordinate or control samples, and pheromones of males who had been previously mounted.	54
	LE	Female preference	~	Mating status (Mated vs virgin) + Calling song (long trill vs short trill)	Previously mated females were less responsive to male calls than virgins, and manipulation of calls had no effect on female preference.	55
	LE	Female preference	~	Age $_{F}$ Call parameters + Age $_{F}$	Older females were more responsive to calls and, generally, females preferred lower pulse rates, but this varied with age.	56

	LE	Female preference	~	Calling song (Song playback vs Silent speaker) Courtship song (Song playback vs Silent speaker)	Females showed a preference for speakers playing calling song, but not for speakers playing courtship song.	57
	LE	Female preference	~	Calling song exposure (long bouts vs short bouts)	Females exposed to calling songs with long bouts mated sooner than those exposed to calling songs with short bouts.	57
Gryllus lineaticeps	LE	Female preference	~	Age $_{M}$ + Acoustic rearing environment (cross-factored treatments)	Female responses were most plastic in young females with respect to differences in rearing environment.	58
	LE	Female preference	~	Calling song (Chirp rate)	Females preferred fast chirp rates over slow chirp rates.	59
	LE	Female preference	~	Calling song (Chirp rate) + Previous acoustic experience	Females responded more strongly to fast chirp rate calling song the second time they were exposed to such calls than the first.	59

	FO	Female fecundity	~	Diet	Mating benefits for females were reduced when mating with males on a high-quality diet, but male diet had no effect on female fecundity	60
	LE	Spermatophore replacement time	~	Diet Calling characteristics	Males who were fed a high-quality diet or who produced lower chirp rates could transfer additional spermatophores sooner than males on a low-quality diet or who produced higher chirp rates.	61
	LE	Female preference	~	Calling song (chirp rate, chirp duration of stimulus)	Female preference was affected by chirp rate but not chirp duration.	62
	LE	Female preference	~	Exposure to high chirp rate call	Female preferences for low chirp rate calls declined following exposure to a high chirp rate call.	63
Gryllus pennsylvanicus	LO	Calling song (Call characteristics) Courtship song (Call characteristics)	~	Body size $_{M}$	Body size is positively associated with calling song amplitude and courtship song pulse amplitude, but negatively associated with chirp rate and carrier frequency.	64

	LE	Relative mating success $_{M}$ (experienced vs inexperienced female)	~	Age $_{M}$ Residual head width Body size $_{M}$ Residual mass	The opportunity for sexual selection was greater when mating with experienced females than inexperienced females.	65
	LO	Calling song (Principal components)	~	Age $_{M}$	Age had a significant effect on a principal component comprised of high values for chirp duration, pulses per chirp and pulse period, and low values of pulse duration and pulse period.	66
	LE	Calling effort	~	Diet	Males fed a high-quality diet spent more time calling each day and lived longer than males fed a low-quality diet.	67
	LE	Female preference	~	Body condition $_{F}$ + Calling effort (high vs low)	Females preferred high calling effort stimuli, independent of their own condition, but high condition females were more choosy than poor condition females.	68

	LE	Probability of mating Latency to copulate	~	Age $_F$ + Mating status $_F$ (cross-factored treatments)	The likelihood of a female mating was independent of treatment, although virgin females mated sooner than mated females. Age had no effect.	69
	LE	Calling duration Search distance	~	Sex ratio (all males, male-biased, equal, female biased)	Calling duration and search distance decreased as the number of females increased.	70
	LE	Female movement Courtship frequency Mating frequency Repeated mating rate	~	Sex ratio	Female movement varied among sex ratios, with the least movement observed in female biased conditions. Female-biased sex ratios had the lowest rates of courtship and remating while male biased sex ratios led to the most matings overall.	71
Gryllus rubens	LE	Female preference	~	Predation risk (spring-emerging female vs autumn-emerging female)	Females who emerged in spring responded to male call playbacks while those emerging in autumn did not respond.	72
Gryllus texensis	LO	Number of signalling males	~	Monitoring treatment (wild-caught vs lab-reared) +	Calling behaviour was increased in populations raised in the lab but independent of monitoring environment.	73

		Time spent signalling		Rearing condition (natural vs lab)	
	LE	Courtship song (Call characteristics)	~	Diet + Life stage	There was no effect of diet, nor the life-stage at which the diet was provided, on courtship song characteristics.	74
	LE	Calling effort	~	Attractiveness $_{M}$ + Immune status	Attractive males called more than unattractive males when immune challenged. There was no difference between immune-challenged and control males in the unattractive group.	75
	LE	Male Attractiveness	~	Body size $_{M}$	Male attractiveness was independent of body size.	75
	LO	Calling song (static components)	~	Body size $_{M}$	Body size is found to influence various call characteristics.	76
	LO	Female preference	~	Calling song (Various components)	Males with a more attractive call carrier frequency spent less time calling.	76
Gryllus veletis	LO	Mating Latency	~	Sperm viability	Mating latency was independent of sperm viability.	77

	LO	Sperm number Sperm viability	~	Calling song (various components) + Body size	Sperm number and viability was independent of calling song characteristics and body size.	77
Gryllus veletis, Gryllus pennsylvanicus	FO	Paired status (Paired vs Solitary)	~	Body size $_{M}$ + Parasite load + Age	Paired males were significantly older and had less parasites than solitary males in *G. veletis*. The likelihood of being paired was independent of body size and parasite load in *G. pennsylvanicus*.	78
Laupala cerasina	LE	Number of females that mated Spermatophore retention time Female remating rate Days until oviposition	~	Nuptial gift provision (provided vs Not provided)	Female reproductive behaviour was independent of nuptial gift provisioning.	79
	LE	Female preference	~	Calling song (Various components)	Females were more responsive to lower pulse rates, lower carrier frequencies, and high pulse durations.	80

Loxoblemmus doenitzi	LO	Calling effort	~	Morphology	No correlation between calling effort and male morphology detected.	81
Meloimorpha japonica	LO	Mean calling duration per day	~	Body mass $_{M}$ + Lifespan + Age (factor)	Calling duration increased with age, but calling effort was independent of body mass.	82
	LE	Female preference	~	Calling song (short vs long interphase duration)	Females preferred short interphase durations over long interphase durations.	83
Nemobius sylvestris	LE	Spermatophore transfer success	~	Nuptial gift provisioning (Forewings intact vs Forewings covered)	Males with covered forewings were less likely to successfully transfer a spermatophore.	84
Neonemobius sp.	LO	Female preference	~	Body size $_{M}$ + Calling effort	Larger males called more often and females preferred larger males in choice experiments.	85
Oecanthus nigricornis	LE	Female fecundity	~	Body size $_{F}$	Larger females were more fecund.	86
	LE	Male competitive ability Female competitive ability	~	Relative competitor size	Mate preferences were independent of body size, but larger males won more fights and females preferred winning males.	86

	LE	Spermatophore size	~	Body size $_M$	Spermatophore size was positively correlated with body size.	86
	LE	Courtship feeding time Spermatophore retention time	~	Diet $_M$ + Diet $_F$	There was a significant interaction between male and female diet on courtship feeding time. The longest feeding times were recorded when a high-quality diet male was paired with a low-quality diet female.	87
	LE	Call pulse duration	~	Body size $_M$	Larger males produced calls which had shorter pulse durations.	88
	LE	Calling song (Carrier frequency)	~	Body size $_M$	Smaller males produced higher frequency calls.	88
	LE	Female preference	~	Calling song (various components)	Females showed no preferences based on pulse duration, and preferred lower frequency calls only when the difference in frequency between calls was large.	88
	LO, LE	Depletion of nuptial gifts	~	Remating	Mating depletes gift reserves but the difference in total residual protein in the gland before and after mating is not significant.	89

	LO, LE	Gland mass Residual protein mass	~	Body size $_M$	There was no effect of body size on residual protein mass.	89
	LO, LE	Mating success	~	Sex ratio (Male bias vs Female bias)	Sex ratio had no effect on mating success, but males in the male-biased treatment transferred larger nuptial gifts.	89
	LE	Female rejection rate	~	Nuptial gift size Body size $_M$	Males depleted of gland proteins were more likely to be rejected, independent of size.	90
Oecanthus nivelus	LE	Female preference	~	Calling song (Large males vs Small males)	More females preferred the large male's call to the small male's call.	91
Ornebius aperta	LO, FO	Mating success	~	Diet + Body size $_M$ + Body mass $_M$	Mating success was independent of diet, but high-quality males transferred more spermatophores than low-quality males, independent of body size.	92
Platygryllus primiformis	LO	Female preference	~	Body size $_M$	Females preferred the larger male	93
	LO	Latency to mount Spermatophore retention time	~	Body size $_M$	Females mated sooner with larger males and retained their spermatophores for longer.	93

	LO	Number of solicitations by male	~	Body size $_F$	In a choice test, males courted both females but preferentially mated with the larger female.	93
Pteronemobious sp.	LE	Attractiveness $_M$ Longevity $_M$	~	Diet	Males on a high-quality diet lived longer and were more attractive than males on a low-quality diet.	94
Teleogryllus commodus	LE	Calling effort Longevity	~	Rival presence (present vs absent)	Rival presence had no effect on calling effort but male longevity was increased in the absence of a rival male.	95
	LE	Calling effort	~	Mating treatment (early, late, multiple, none)	Mating treatment had no effect on calling effort, but future investment in calling was reduced in mated males.	96
	LE	Latency to call Latency to mount Spermatophore retention time	~	Immune activation treatment + Mating status	Immune challenge had no effect on male attractiveness. Mating status had no effect on latency to mate but virgins took longer to remove spermatophores than previously mated females.	97

	LE	Calling effort Longevity	~	Diet	Increased dietary protein reduced male longevity but increased calling effort. Increased dietary protein increased female longevity.	98
	LE	Hatching success Offspring survival	~	Number of mates	There was no effect of the number of times a female mated on hatching success or offspring survival. There was a small decline in hatching success for each subsequent male.	99
	LO	Mating success	~	Attractiveness $_{M}$ Fighting ability	Attractiveness was independent of fighting ability. Body size had no direct effect on attractiveness, but larger males were better fighters. Mating success was greater for attractive males and fight winners.	100
	LE	Insemination success	~	Attractiveness $_{M}$ + Body size $_{F}$	Insemination success was increased for more attractive males and smaller females.	101
Teleogryllus natalensis	LO	Spermatophore retention time	~	Rival presence + Mating male presence (cross-factored treatments)	Rival presence had no effect on spermatophore retention time, while guard presence had a positive effect on spermatophore retention time.	102

Teleogryllus oceanicus	LE	Female preference ~ Call song (proportion of long chirps) + Body Size $_M$	Female preference was independent of male body size. The number of female responses favours directional selection for a high proportion of long trills in male calls, while response effort favours stabilising selection on an intermediate number of trills.	103
	LE	Female preference (Calling song) ~ Cuticular Hydrocarbon Exposure	Female preference for calling song was independent of cuticular hydrocarbon exposure.	104
	LE	Female preference (Calling song) ~ Acoustic rearing environment	Females reared in silence were more responsive to male calling song, while those previously exposed to calling song were choosier.	105
	LE	Female preference ~ Calling song (proportion of long chirps) + Previous acoustic experience	Females generally preferred calls with more long chirps, however females previously exposed to a preferred call showed weaker responses than females previously exposed to less attractive calls.	106

	LE	Female preference (Calling song) Reproductive tissue mass Condition	~	Acoustic rearing environment	Males raised in silence were more likely to exhibit satellite behaviour, invested less in reproductive tissue mass, and attained worse adult condition than those exposed to conspecific song.	107
	LE	Mating Latency	~	Courtship song (Amplitude) + Mating status	Latency to mount was independent of courtship song amplitude, but virgin females mated significant faster than previously mated females.	108
	LE	Sperm viability Paternity	~	Acoustic rearing environment	Males reared in silence produced spermatophores with a lower proportion of viable sperm than those exposed to conspecific song, however paternity share was independent of rearing environment.	109
	LE	Female preference	~	Acoustic rearing environment	Female preference is independent of acoustic environment during rearing.	110
	LE	Female reproductive investment	~	Acoustic rearing environment	Acoustic rearing environment had no effect on female reproductive investment.	110

	LO	Latency to mount	~	Treatment group (preferred male with non-preferred courtship call vs non-preferred male with preferred courtship call)	Females preferred males who called with a higher duty cycle and whose songs were longer. While preferred males with preferred songs were more attractive to females than non-preferred males with preferred songs, a non-preferred male could be made attractive by playing the preferred song. There was no difference between preferred males with non-preferred songs and non-preferred males with non-preferred songs.	111
	LO	Female preference	~	Courtship song (principal components) + Cuticular hydrocarbon profile	Females preferred courtship songs with short trills and many long pulses with short intervals between pulses. Females also preferred distinct cuticular hydrocarbon profiles, with males expressing average cuticular hydrocarbon profiles achieving the lowest mating success.	112
	LE	Paternity share	~	Sperm number + Sperm length	Neither sperm number nor sperm length had a significant effect on paternity.	113

	LE	Cuticular Hydrocarbon expression	~	Fighting ability	Winning and losing males expressed different cuticular hydrocarbon profiles.	114
	LE	Paternity share	~	Dominance rank $_M$	Dominant males sired significantly more offspring when competing directly with subordinate males.	114
	LE	Sperm viability	~	Dominance rank $_M$ + Body mass $_M$ + Body size $_M$	The ejaculates of dominant males contained more viable sperm than those of subordinate males, independent of body size and body mass.	114
	LE	Cuticular Hydrocarbon expression	~	Acoustic rearing environment	Males reared in silence expressed different cuticular hydrocarbon profiles than those reared in the presence of conspecific calling song.	115
	LE	Spermatophore production time Sperm viability	~	Female familiarity (familiar vs novel female)	Rate of spermatophore production and sperm viability were independent of female familiarity.	116

Appendix 2b: Table of references for studies featured in appendix 2a

Table of references cited in appendix 2a including author(s), year or publication and short title. Full references are given in **Bibliography**.

Ref.	Citation
1	Assis, Trietsch & Foellmer, 2017, Male mate choice based on chemical cues in the cricket *Acheta domesticus* (Orthoptera: Gryllidae)
2	Bertram, *et al*., 2011, Variation in cricket acoustic mate attraction signalling explained by body morphology and metabolic differences
3	Bertram, *et al*., 2009, Phosphorus availability influences cricket mate attraction displays
4	Fleischman & Sakaluk, 2004, No direct or indirect benefits to cryptic female choice in house crickets (*Acheta domesticus*)
5	Gray, 1997, Female house cricket, *Acheta domesticus*, prefer the chirps of larger males
6	Gray, 1999, Intrinsic factors affecting female choice in house crickets
7	Mautz & Sakaluk, 2008, The effects of age and previous mating experience on Pre- and Post-copulatory mate choice in female house crickets (*Acehta domesticus* L.)
8	Nelson & Nolen, 1997, Courtship song, male agonistic encounters, and female mate choice in the house cricket, *Acheta domesticus* (Orthoptera: Gryllidae)
9	Reinhardt & Siva-Jothy, 2005, An advantage for young sperm in the house cricket *Acheta domesticus*
10	Ryder, 2000, Male calling song provides a reliable signal of immune function in a cricket
11	Savage, *et al*., 2005, Male attractiveness covaries with fighting ability but not with prior outcome in house crickets
12	Stoffer & Walker, 2012, The use of multimodal communication in mate choice decisions by female house crickets, *Acheta domesticus*
13	DiRienzo & Marshall, 2013, Function of the hemolymph nuptial gift in the ground cricket, *Allonemobius socius*

14	Fedorka & Mousseau, 2002, Tibial spur feeding in ground crickets
15	Fedorka & Mousseau, 2007, Immune system activations affects male sexual signal and reproductive potential in crickets
16	Fedorka, *et al*., 2004, Immune suppression and the cost of reproduction in the ground cricket, *Allonemobius socius*
17	Olvido & Wagner, 2004, Signal components, acoustic preference functions and sexual selection in a cricket
18	Sadowski, Grace & Moore, 2002, Complex courtship behaviour in the striped ground cricket, *Allonemobius socius* (Orthoptera: Gryllidae)
19	Bateman & MacFadyen, 1999, Mate guarding in the cricket *Gryllodes sigillatus*
20	Champagnon & del Castillo, 2008, Female mate choice, calling song and genetic variance in the cricket, *Gryllodes sigillatus*
21	Gershman & Sakaluk, 2010, Mate quality and novelty influence post-copulatory female choice in decorated crickets, *Gryllodes sigilatus*
22	Ivy & Sakaluk, 2006, Sequential mate choice in decorated crickets
23	Ketola, Kortet & Kotiaho, 2007, Testing theories of sexual selection in decorated crickets (*Gryllodes sigillatus*)
24	Ketola, Kortet & Kotiaho, 2009, Endurance in exercise is associated with courtship call rate in decorated crickets, *Gryllodes sigillatus*
25	Ritz & Sakaluk, 2002, The role of the male's cerci in copulation and mate guarding in decorated crickets (*Gryllodes sigillatus*)
26	Schaus & Sakaluk, 2001, Ejaculate expenditures of male crickets in response to varying risk and intensity of sperm competition
27	Sakaluk, 1991, Post-copulatory mate guarding in the decorated crickets
28	Bertram et al., 2016, Linking mating preferences to sexually selected traits and offspring viability
29	Bertram, *et al*., 2017, What is driving male mate preference evolution in Jamaican field crickets?
30	Loranger & Bertram, 2016, The effect of male dominance on female choice in a field cricket (*Gryllus assimilis*)
31	Pacheco & Bertram, 2014, How male sound pressure level influence phonotaxis in virgin female Jamaican field crickets (*Gryllus assimilis*)

32	Vedenina & Pollack, 2012, Recognition of variable courthship song in the field cricket *Gryllus assimilis*
33	Whattam & Bertram, 2011, Effects of juvenile and adult condition on long-distance call components in the Jamaican field cricket, *Gryllus assimilis*
34	Bateman, 1998, Mate preference for novel partners in the cricket *Gryllus bimaculatus*
35	Bateman & Flemming, 2006, Males are selective too
36	Bateman, Gilson & Ferguson, 2001, Male size and sequential mate preferences in the cricket *Gryllus bimaculatus*
37	Bretman, Rodriguez-Munoz & Tregenza, 2006, Male dominance determines female egg laying rate in crickets
38	Green & Tregenza, 2008, The influence of male ejaculates on female mate search behvaviour, oviposition and longevity in crikets
39	Rantala & Kortet, 2003, Courtship song and immune function in the field cricket *Gryllus bimaculatus*
40	Simmons, 1987, Female choice contributes to offspring fitness in the field cricket, *Gryllus bimaculatus* (De Geer)
41	Simmons & Zuk, 1992, Variability in call structure and pairing success of male field cricket, *Gryllus bimaculatus*
42	Tregenza & Wedell, 1998, Benefits of multiple mates in the cricket *Gryllus bimaculatus*
43	Verburgt & Ferguson, 2010, Mate choice in field crickets
44	Verburgt, Ferreira & Ferguson, 2010, Male field cricket song reflects age, allowing females to prefer young males
45	Wedell & Tregenza, 1999, Successful fathers sire successful sons
46	Wynn & Vahed, 2004, Male *Gryllus bimaculatus* guard females to delay them from mating with rival males and to obtain repeated copulations
47	Zhemchuzhnikov, *et al*., 2017, Louder songs can enhance attractiveness of old male crickets (*Gryllus bimaculatus*)
48	Holzer *et al*., 2003, Condition-dependent signaling affects male sexual attractiveness in field crickets, *Gryllus campestris*
49	Jacot, *et al*., 2005, Juvenile immune status affects the expression of a sexually selected trait in field crickets

50	Ritz & Kohler, 2010, Natural and sexual selection on male behaviour and morphology, and female choice in a wild field cricket population
51	Simmons, 1995, Correlates of male quality in the field cricket, *Gryllus campestris* L
52	Saleh, Larson & Harrison, 2014, Reproductive success and body size in the cricket *Gryllus firmus*
53	Cade & Cade, 1992, Male mating success, calling and searching behaviour at high and low-densities in the field cricket, *Gryllus integer*
54	Kortet & Hedrick, 2005, The scent of dominance
55	Lickman, Murray & Cade, 1998, Effect of mating on female phonotactic response in *Gryllus integer* (Orthoptera: Gryllidae)
56	Prosser, Murray & Cade, 1997, The influence of female age on phonotaxis during single and multiple song presentations in the field cricket, *Gryllus integer* (Orthoptera: Gryllidae)
57	Leonard & Hedrick, 2010, Long-distance signals influence assessment of close range mating displays in the field cricket, *Gryllus integer*
58	Atwell & Wagner, 2014, Female mate choice plasticity is affected by the interaction between male density and female age in a field cricket
59	Beckers & Wagner, 2011, Mate sampling strategy in a field cricket
60	Tolle & Wagner, 2011, Costly signals in a field cricket can indicate high- or low-quality direct benefits depending upon the environment
61	Wagner, 2005, Male field crickets that provide reproductive benefits to females incur higher costs
62	Wagner & Basolo, 2006, The relative importance of different direct benefits in the mate choices of a field cricket
63	Wagner, Smeds & Wiegmann, 2001, Experience affects female responses to male song in the variable field cricket *Gryllus lineaticeps* (Orthoptera, Gryllidae)
64	Harrison, *et al*., 2013, Calling, courtship and condition in the fall field cricket, *Gryllus pennsylvanicus*
65	Judge, 2010, Female social experience affects the shape of sexual selection on males

66	Judge, 2011, Do male field crickets, *Gryllus pennsylvanicus*, signal their age?
67	Judge, Ting & Gwynne, 2008, Condition dependence of male life span and calling effort in a field cricket
68	Judge, Ting & Gwynne, 2014, Condition dependence of female choosiness in a field cricket
69	Judge, Tran & Gwynne, 2010, The relative effects of mating status and age on the mating behaviour of female field crickets
70	Souroukis & Cade, 1993, Reproductive competition and selection on male traits at varying sex-ratios in the field cricket, *Gryllus pennsylvanicus*
71	Souroukis & Murray, 1995, Female mating behaviour in the field cricket, *Gryllus pennsylvanicus* (Orthoptera, Gryllidae) at different operational sex ratios
72	Velez & Brockmann, 2006, Seasonal variation in female response to male calling song in the field cricket, *Gryllus rubens*
73	Bertram, 2002, The influence of rearing and monitoring environment on temporal mate signalling patterns in the field cricket, *Gryllus texensis*
74	Gray & Eckhardt, 2001, Is cricket courtship song condition dependent?
75	Kelly, Telemeco & Bartholomay, 2015, Are attractive male crickets better able to pay the costs of an immune challenge?
76	McAuley & Bertram, 2016, Field Crickets compensate for unattractive static long-distance call components by increasing dynamic signalling effort
77	Fitzsimmons & Bertram, 2013, No relationship between long-distance acoustic mate attraction signals and male fertility or female preference in spring field crickets
78	Zuk, 1988, Parasite load, body size, and age of wild-caught male field crickets (Orthoptera, Gryllidae)
79	Shaw, 2009, Elaborate courtship enhances sperm transfer in the Hawaiian swordtail cricket, *Laupala cerasina*
80	Shaw & Herlihy, 2000, Acoustic preference functions and song variability in the Hawaiian cricket *Laupala cerasina*
81	Kuriwada, 2016, Horn length is not correlated with calling efforts in the horn-headed cricket *Loxoblemmus doenitzi* (Orthoptera: Gryllidae)

82	Kuriwada & Kasuya, 2011, Age-dependent changes in calling effort in the bell cricket *Meloimorpha japonica*
83	Kuriwada & Kasuya, 2006, Female responsiveness is negatively correlated with life expectancy in *Meloimorpha japonica* (Orthoptera: Gryllidae)
84	Prokop & Maxwell, 2008, Interactions between multiple forms of nuptial feeding in the wood cricket *Nemobius sylvestris* (Bosc)
85	Forrest, *et al*., 1991, Mate choice in ground crickets (Gryllidae, nemoiinae)
86	Brown, 2008, Size-biased mating in both sexes of the black-horned tree cricket, *Oecanthus nigricornis* Walker (Orthoptera: Gryllidae)
87	Brown, 2011, Allocation of nuptial gifts in tree crickets changes with both male and female diet
88	Brown, *et al*., 1996, Female choice for an indicator of male size in the song of the black-horned tree cricket *Oecantus nigricornis* (Orthoptera: Gryllidae: Oecanthinae)
89	Bussiere, Basit & Gwynne, 2005, Preferred males are not always good providers female choice and male investment in tree crickets
90	Bussiere, Clark & Gwynne, 2005, Precopulatory choice for cues of material benefits in tree crickets
91	Ponce-Wainer & del Castillo, 2008, Female mate choice and no detected predation risk in relation to the calling song of *Oecanthus nivelus* (Gryllidae: oecanthinae)
92	Andrade & Mason, 2000, Male condition, female choice, and extreme variation in repeated mating in a scaly cricket, *Ornebius aperta* (Orthoptera: Gryllidae: mogoplistinae)
93	Bateman, 1998, Assortative mating by both sexes in the cricket *Platygryllus primiformis* (Orthoptera: gryllidae; gryllinae)
94	Hall, Bussiere & Brooks, 2008, The effect of diet quality and wing morph on male and female reproductive investment in a nuptial feeding ground cricket
95	Callander, *et al*., 2013, The effect of competitors on calling effort and life span in male field crickets
96	Chiswell, *et al*., 2014, Prior mating success can affect allocation towards future sexual signalling in crickets

97	Drayton, Boeke & Jennions, 2012, Immune challenge and pre- and post-copulatory female choice in the cricket *Teleogryllus commodus*
98	Hunt, *et al*., 2004, High-quality male field crickets invest heavily in sexual display but die young
99	Jennions, *et al*., 2007, Do female black field crickets *Teleogryllus commodus* benefit from polyandry
100	Shackleton, Jennions & Hunt, 2005, Fighting success and attractiveness as predictors of male mating success in the black field cricket, *Teleogryllus commodus*: the effectiveness of no-choice tests
101	Hall *et al*., 2010, Competitive PCR reveals the complexity of post-copulatory sexual selection in *Teleogryllus commodus*
102	Hockham & Vahed, 1997, The function of mate guarding in a field cricket (Orthoptera: Gryllidae; *Teleogryllus natalensis* Otte and Cade)
103	Bailey, 2008, Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets
104	Bailey, 2011, Mate choice plasticity in the field cricket *Teleogryllus oceanicus*
105	Bailey & Zuk, 2008, Acoustic experience shapes female mate choice in field crickets
106	Bailey & Zuk, 2009, Field crickets change mating preferences using remembered social information
107	Bailey, Gray & Zuk, 2010, Acoustic experience shapes alternative mating tactics and reproductive investment in male field crickets
108	Balenger & Zuk, 2017, Relative amplitude of courtship song chirp and trill components does not alter female *Teleogryllus oceanicus* mating behaviour
109	Gray & Simmons, 2013, Acoustic cues alter perceived sperm competition risk in the field cricket *Teleogryllus oceanicus*
110	Lierheimer & Tinghitella, 2017, Quantity and quality of available mates alters female responsiveness but not investment in the pacific field cricket, *Teleogryllus oceanicus*
111	Rebar, Bailey & Zuk, 2009, Courtship song's role during female mate choice in the field cricket *Teleogryllus oceanicus*

112	Simmons, *et al*., 2013, Female preferences for acoustic and olfactory signals during courtship: male crickets send multiple messages
113	Simmons, *et al*., 2003, Variation in paternity in the field cricket *Teleogryllus oeanicus*
114	Thomas & Simmons, 2009, Male dominance influences pheromone expression, ejaculate quality, and fertilization success in the Australian field cricket, *Teleogryllus oceanicus*
115	Thomas, Gray & Simmons, 2011, Male crickets alter the relative expression of cuticular hydrocarbons when exposed to different acoustic environments
116	Thomas & Simmons, 2015, No coolidge effect in the Australian field cricket *Teleogryllus oceanicus* (orthoptera: Gryllidae)

Bibliography

Abrahams, M. V.; Robb, T. L. & Hare, J. F. 2005. Effect of hypoxia on opercular displays: Evidence for an honest signal? *Animal Behaviour,* 70**,** 427-432.

Abrams, P. A.; Leimar, O.; Nylin, S. & Wiklund, C. 1996. The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. *The American Naturalist,* 147**,** 381-395.

Alexander, R. D. 1961. Aggressiveness, territoriality, and sexual behavior in field crickets (orthoptera: Gryllidae). *Behaviour***,** 130-223.

Almbro, M. & Simmons, L. W. 2014. Sexual selection can remove an experimentally induced mutation load. *Evolution,* 68**,** 295-300.

Andersson, M. B. 1994. *Sexual selection,* Princeton, New Jersey, Princeton University Press.

Andrade, M. C. B. & Mason, A. C. 2000. Male condition, female choice, and extreme variation in repeated mating in a scaly cricket, *Ornebius aperta* (Orthoptera : Gryllidae : Mogoplistinae). *Journal of Insect Behavior,* 13**,** 483-497.

Aquiloni, L.; Buřič, M. & Gherardi, F. 2008. Crayfish females eavesdrop on fighting males before choosing the dominant mate. *Current Biology,* 18**,** R462-R463.

Aquiloni, L. & Gherardi, F. 2010. Crayfish females eavesdrop on fighting males and use smell and sight to recognize the identity of the winner. *Animal Behaviour,* 79**,** 265-269.

Arbuthnott, D. & Rundle, H. D. 2012. Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*. *Evolution,* 66**,** 2127-2137.

Arbuthnott, D. & Rundle, H. D. 2014. Misalignment of natural and sexual selection among divergently adapted drosophila melanogaster populations. *Animal Behaviour,* 87**,** 45-51.

Archer, C.R.; Royle, N.; South, S.; Selman, C.; Hunt, J. 2009. Nutritional geometry provides food for thought. *J Gerontol. A Biol. Sci. Med. Sci*, 64A, 956-959.

Arnott, G. & Elwood, R.W. 2009. Assessment of fighting ability in animal contests. *Animal behaviour*, 77, 991-1004

Assis, B. A.; Trietsch, C. & Foellmer, M. W. 2017. Male mate choice based on chemical cues in the cricket acheta domesticus (orthoptera: Gryllidae). *Ecological Entomology,* 42**,** 11-17.

Attard, L. 2013. *The development and evaluation of a gut-loading diet for feeder crickets formulated to provide a balanced nutrient source for insectivorous amphibians and reptiles.* Master of Science, University of Guelph.

Atwell, A. & Wagner, W. E. 2014. Female mate choice plasticity is affected by the interaction between male density and female age in a field cricket. *Animal Behaviour,* 98**,** 177-183.

Atwell, A. & Wagner, W. E. 2015. Along came a spider who sat down beside her: Perceived predation risk, but not female age, affects female mate choosiness. *Behavioural Processes,* 115**,** 143-148.

Bailey, N. W. 2008. Love will tear you apart: Different components of female choice exert contrasting selection pressures on male field crickets. *Behavioral Ecology,* 19**,** 960-966.

Bailey, N. W. 2011. Mate choice plasticity in the field cricket teleogryllus oceanicus: Effects of social experience in multiple modalities. *Behavioral Ecology and Sociobiology,* 65**,** 2269-2278.

Bailey, N. W.; Gray, B. & Zuk, M. 2010. Acoustic experience shapes alternative mating tactics and reproductive investment in male field crickets. *Current Biology,* 20**,** 845-849.

Bailey, N. W. & Zuk, M. 2008. Acoustic experience shapes female mate choice in field crickets. *Proceedings of the Royal Society of London B: Biological Sciences,* 275**,** 2645-2650.

Bailey, N. W. & Zuk, M. 2009. Field crickets change mating preferences using remembered social information. *Biology letters,* 5**,** 449-451.

Baker, M. B.; Venugopal, P. D. & Lamp, W. O. 2015. Climate change and phenology: Empoasca fabae (hemiptera: Cicadellidae) migration and severity of impact. *Plos One,* 10**,** 1-12.

Balenger, S. L.; Lara, L. M. & Zuk, M. 2017. Relative amplitude of courtship song chirp and trill components does not alter female teleogryllus oceanicus mating behavior. *Ethology,* 123**,** 168-173.

Ballentine, B.; Hyman, J.; Nowicki, S. 2004. Vocal performance influences female response to male bird song: an experimental test. *Behavioural Ecology*, 15, 163-168.

Banks, M. J. & Thompson, D. J. 1985. Lifetime mating success in the damselfly coenagrion puella. *Animal Behaviour,* 33**,** 1175-1183.

Barbosa, P.; Martinat, P. & Waldvogel, M. 1986. Development, fecundity and survival of the herbivore lymantria dispar and the number of plant species in its diet. *Ecological Entomology,* 11**,** 1-6.

Barnett, S. A. 1958. Experiments on ‘neophobia’in wild and laboratory rats. *British journal of psychology,* 49**,** 195-201.

Barton, K. 2016. Mumin: Multi-model inference.

Bateman, P. W. 1998a. Assortative mating by both sexes in the cricket platygryllus primiformis (orthoptera : Gryllidae; gryllinae). *Transactions of the American Entomological Society,* 124**,** 63-68.

Bateman, P. W. 1998b. Mate preference for novel partners in the cricket gryllus bimaculatus. *Ecological Entomology,* 23**,** 473-475.

Bateman, P. W. & Fleming, P. A. 2006. Males are selective too: Mating, but not courtship, with sequential females influences choosiness in male field crickets (gryllus bimaculatus). *Behavioral Ecology and Sociobiology,* 59**,** 577-581.

Bateman, P. W.; Gilson, L. N. & Ferguson, J. W. H. 2001. Male size and sequential mate preference in the cricket gryllus bimaculatus. *Animal Behaviour,* 61**,** 631-637.

Bateman, P. W. & Macfadyen, D. N. 1999. Mate guarding in the cricket gryllodes sigillatus: Influence of multiple potential partners. *Ethology,* 105**,** 949-957.

Bates, D.; Maechler, M.; Boler, B. & Walker, S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software,* 67**,** 1-48.

Beckers, O. M. & Wagner Jr, W. E. 2011. Mate sampling strategy in a field cricket: Evidence for a fixed threshold strategy with last chance option. *Animal Behaviour,* 81**,** 519-527.

Beckers, O. M. & Wagner, W. E. 2013. Parasitoid infestation changes female mating preferences. *Animal Behaviour,* 85**,** 791-796.

Bennet-Clark, H. C. 1989. Songs and the physics of sound production. *In:* HUBER, F., MOORE, T. E. & LOHER, W. (eds.) *Cricket behavior and neurobiology.* Ithaca, NY: Cornell University Press.

Benton, T. 2012. *Grasshoppers & crickets,* London, HarperCollins Publishers.

Berger, D.; Walters, R. & Gotthard, K. 2006. What keeps insects small?—size dependent predation on two species of butterfly larvae. *Evolutionary Ecology,* 20**,** 575-589.

Berglund, A. & Rosenqvist, G. 2001. Male pipefish prefer dominant over attractive females. *Behavioral Ecology,* 12**,** 402-406.

Bertram, S.; Thomson, I.; Auguste, B.; Dawson, J. & Darveau, C.-A. 2011. Variation in cricket acoustic mate attraction signalling explained by body morphology and metabolic differences. *Animal behaviour,* 82**,** 1255-1261.

Bertram, S. M. 2002. The influence of rearing and monitoring environment on temporal mate signaling patterns in the field cricket, gryllus texensis. *Journal of Insect Behavior,* 15**,** 127-137.

Bertram, S. M.; Harrison, S. J.; Ferguson, G. L.; Thomson, I. R.; Loranger, M. J.; Reifer, M. L.; Corlett, D. H. & Gowaty, P. A. 2017. What is driving male mate preference evolution in jamaican field crickets? *Ethology,* 123**,** 793-799.

Bertram, S. M.; Loranger, M. J.; Thomson, I. R.; Harrison, S. J.; Ferguson, G. L.; Reifer, M. L.; Corlett, D. H. & Gowaty, P. A. 2016. Linking mating preferences to sexually selected traits and offspring viability: Good versus complementary genes hypotheses. *Animal Behaviour,* 119**,** 75-86.

Bertram, S. M.; Whattam, E. M.; Visanuvimol, L.; Bennett, R. & Lauzon, C. 2009. Phosphorus availability influences cricket mate attraction displays. *Animal Behaviour,* 77**,** 525-530.

Birkhead, T.R., Fletcher, F. & Pellatt, E. J. (1998). Sexual selection in the zebra finch Taeniopygia guttata : condition, sex traits and immune capacity. *Behavioral Ecology and Sociobiology*, 44, 179–191.

Blakley, N. & Goodner, S. R. 1978. Size-dependent timing of metamorphosis in milkweed bugs (oncopeltus) and its life-history implications. *Biological Bulletin,* 155**,** 499-510.

Blanckenhorn, W. U. 2000. The evolution of body size: What keeps organisms small? *The quarterly review of biology,* 75**,** 385-407.

Blanckenhorn, W. U.; Preziosi, R. F. & Fairbairn, D. J. 1995. Time and energy constraints and the evolution of sexual size dimorphism—to eat or to mate? *Evolutionary Ecology,* 9**,** 369-381.

Blomqvist, D.; Johansson, O. C.; Unger, U.; Larsson, M. & Flodin, L.-Å. 1997. Male aerial display and reversed sexual size dimorphism in the dunlin. *Animal Behaviour,* 54**,** 1291-1299.

Blows, M. W. 2002. Interaction between natural and sexual selection during the evolution of mate recognition. *Proceedings of the Royal Society B-Biological Sciences,* 269**,** 1113-1118.

Boggs, C.L. & Freeman K.D. 2005. Larvalfood limitation in butterflies: effects on adult resource allocation and fitness. *Oecologia*, 144, 353-361.

Both, C.; Artemyev, A. V.; Blaauw, B.; Cowie, R. J.; Dekhuijzen, A. J.; Eeva, T.; Enemar, A.; Gustafsson, L.; Ivankina, E. V.; Jarvinen, A.; Metcalfe, N. B.; Nyholm, N. E. I.; Potti, J.; Ravussin, P. A.; Sanz, J. J.; Silverin, B.; Slater, F. M.; Sokolov, L. V.; Torok, J.; Winkel, W.; Wright, J.; Zang, H. & Visser,

M. E. 2004. Large-scale geographical variation confirms that climate change causes birds to lay earlier. *Proceedings of the Royal Society B-Biological Sciences,* 271**,** 1657-1662.

Both, C. & Visser, M. E. 2001. Adjustment to climate change is constrained by arrival date in a long-distance migrant bird. *Nature,* 411**,** 296-298.

Branson, D.H. 2004, Relative importance of nymphal and adult resource availability for reproductive allocation in *Melanoplus sanguinipes* (Orthoptera: Acrididae). *Journal of Orthoptera Research*, 13, 239-245.

Bretman, A.; Gage, M. J. & Chapman, T. 2011. Quick-change artists: Male plastic behavioural responses to rivals. *Trends in ecology & evolution,* 26**,** 467-473.

Bretman, A.; Rodríguez-Muñoz, R. & Tregenza, T. 2006. Male dominance determines female egg laying rate in crickets. *Biology letters,* 2**,** 409-411.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. *Nature,* 406**,** 67-70.

Brooks, R. 2002. Variation in female mate choice within guppy populations: Population divergence, multiple ornaments and the maintenance of polymorphism. *Genetica,* 116**,** 343-358.

Brooks, R. & Endler, J. A. 2001. Female guppies agree to differ: Phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution,* 55**,** 1644-1655.

Brown, W. D. 1990. Size-assortative mating in the blister beetle lytta magister (coleoptera, meloidae) is due to male and female preference for larger mates. *Animal Behaviour,* 40**,** 901-909.

Brown, W. D. 1997. Female remating and the intensity of female choice in black-horned tree crickets, oecanthus nigricornis. *Behavioral Ecology,* 8**,** 66-74.

Brown, W. D. 2008. Size-biased mating in both sexes of the black-horned tree cricket, oecanthus nigricornis walker (orthoptera : Gryllidae : Oecanthinae). *Journal of Insect Behavior,* 21**,** 130-142.

Brown, W. D. 2011. Allocation of nuptial gifts in tree crickets changes with both male and female diet. *Behavioral Ecology and Sociobiology,* 65**,** 1007-1014.

Brown, W. D.; Wideman, J.; Andrade, M. C. B.; Mason, A. C. & Gwynne, D. T. 1996. Female choice for an indicator of male size in the song of the black-horned tree cricket oecanthus nigricornis (orthoptera: Gryllidae: Oecanthinae). *Evolution,* 50**,** 2400-2411.

Buchanan, K. L. & Catchpole, C. K. 2000. Song as an indicator of male parental effort in the sedge warbler. *Proceedings of the Royal Society B-Biological Sciences,* 267**,** 321-326.

Bunning, H.; Bassett, L.; Clowser, C.; Rapkin, J.; Jensen, K.; House, C. M.; Archer, C. R. & Hunt, J. 2016. Dietary choice for a balanced nutrient intake increases the mean and reduces the variance in the reproductive performance of male and female cockroaches. *Ecology and evolution,* 6**,** 4711-4730.

Burley, N. T. & Foster, V. S. 2006. Variation in female choice of mates: Condition influences selectivity. *Animal Behaviour,* 72**,** 713-719.

Bussiere, L. F.; Basit, H. A. & Gwynne, D. T. 2005a. Preferred males are not always good providers: Female choice and male investment in tree crickets. *Behavioral Ecology,* 16**,** 223-231.

Bussiere, L. F.; Clark, A. P. & Gwynne, D. T. 2005b. Precopulatory choice for cues of material benefits in tree crickets. *Behavioral Ecology,* 16**,** 255-259.

Cabral, L. G. & Holland, B. 2014. Courtship song does not increase the rate of adaptation to a thermally stressful environment in a drosophila melanogaster laboratory population. *Plos One,* 9**,** 1-6.

Cade, W. 1975. Acoustically orienting parasitoids: Fly phonotaxis to cricket song. *Science,* 190**,** 1312-1313.

Cade, W. 1979. The evolution of alternative male reproductive strategies in field crickets. *In:* BLUM, M. & BLUM, N. (eds.) *Sexual selection and reproductive competition in insects.* New York, New York: Academic Press, Inc.

Cade, W. H. & Cade, E. S. 1992. Male mating success, calling and searching behaviour at high and low densities in the field cricket, gryllus integer. *Animal Behaviour,* 43**,** 49-56.

Callander, S.; Kahn, A. T.; Hunt, J.; Backwell, P. R. & Jennions, M. D. 2013. The effect of competitors on calling effort and life span in male field crickets. *Behavioral Ecology,* 24**,** 1251-1259.

Candolin, U. 2003. The use of multiple cues in mate choice. *Biological Reviews,* 78**,** 575-595.

Candolin, U. & Heuschele, J. 2008. Is sexual selection beneficial during adaptaiton to environmental change? *Trends in Ecology and Evolution,* 23.

Cardillo, M.; Mace, G. M.; Jones, K. E.; Bielby, J.; Bininda-Emonds, O. R.; Sechrest, W.; Orme, C. D. L. & Purvis, A. 2005. Multiple causes of high extinction risk in large mammal species. *Science,* 309**,** 1239-1241.

Carlson, S. M.; Olsen, E. M. & Vøllestad, L. A. 2008. Seasonal mortality and the effect of body size: A review and an empirical test using individual data on brown trout. *Functional Ecology,* 22**,** 663-673.

Chaine, A.S. & Lyon, B.E. 2008. Adaptive plasticity in female mate choice dampens sexual selection on male ornaments in the lark bunting. *Science*, 319, 459-462.

Champagnon, J. & Del Castillo, R. C. 2008. Female mate choice, calling song and genetic variance in the cricket, gryllodes sigillatus. *Ethology,* 114**,** 223-230.

Charlwood, J.; Pinto, J.; Sousa, C.; Ferreira, C. & Do Rosario, V. 2002. Male size does not affect mating success (of anopheles gambiae in sao tome). *Medical and veterinary entomology,* 16**,** 109-111.

Chen, I. C.; Hill, J. K.; Ohlemuller, R.; Roy, D. B. & Thomas, C. D. 2011. Rapid range shifts of species associated with high levels of climate warming. *Science,* 333**,** 1024-1026.

Chenoweth, Stephen f.; Appleton, Nicholas c.; Allen, Scott l. & Rundle, Howard d. 2015. Genomic evidence that sexual selection impedes adaptation to a novel environment. *Current Biology,* 25**,** 1860-1866.

Chiswell, R.; Girard, M.; Fricke, C. & Kasumovic, M. M. 2014. Prior mating success can affect allocation towards future sexual signaling in crickets. *PeerJ,* 2**,** 1-13.

Ciceran, M.; Murray, A.M.; Rowell, G. 1994. Natural variation in the temporal patterning of calling song structure in the field cricket *Gryllus pennsylvanicus*: effects of temperature, age, mass, time of day, and nearest neighbour. *Canadian journal of Zoology*, 72, 38-42.

Clark, R. W.; Schuett, G. W.; Repp, R. A.; Amarello, M.; Smith, C. F. & Herrmann, H.-W. 2014. Mating systems, reproductive success, and sexual selection in secretive species: A case study of the western diamond-backed rattlesnake, crotalus atrox. *PLoS One,* 9**,** 1-12.

Clifford, C. W. & Woodring, J. P. 1990. Methods for rearing the house cricket, acheta domesticus (l) along with baseline values for feeding rates, growth rates, development times, and blood composition. *Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie,* 109**,** 1-14.

Clutton-Brock, T. H. 1988. *Reproductive success: Studies of individual variation in contrasting breeding systems*, University of Chicago Press.

Clutton-Brock, T. H. & Albon, S. D. 1979. Roaring of red deer and the evolution of honest advertisement. *Behaviour,* 69**,** 145-170.

Clutton-Brock, T. H.; Albon, S. D. & Guinness, F. E. 1985. Parental investment and sex differences in juvenile mortality in birds and mammals. *Nature,* 313**,** 131-133.

Coleman, S. W.; Patricelli, G. L. & Borgia, G. 2004. Variable female preferences drive complex male displays. *Nature,* 428**,** 742-745.

Cotton, S.; Fowler, K. & Pomiankowski, A. 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society B: Biological Sciences,* 271**,** 771-783.

Cotton, S.; Rogers, D. W.; Small, J.; Pomiankowski, A. & Fowler, K. 2006a. Variation in preference for a male ornament is positively associated with female eyespan in the stalk-eyed fly diasemopsis meigenii. *Proceedings of the Royal Society of London B: Biological Sciences,* 273**,** 1287-1292.

Cotton, S.; Small, J. & Pomiankowski, A. 2006. Sexual selection and condition-dependent mate preferences. *Current Biology,* 16**,** R755-R765.

Couvillon, M. J.; Hughes, W. O.; Perez-Sato, J. A.; Martin, S. J.; Roy, G. G. & Ratnieks, F. L. 2010. Sexual selection in honey bees: Colony variation and the importance of size in male mating success. *Behavioral Ecology,* 21**,** 520-525.

Cratsley, C. K. & Lewis, S. M. 2003. Female preference for male courtship flashes in photinus ignitus fireflies. *Behavioral Ecology,* 14**,** 135-140.

Crawley, M. J. 2005. *Statistics: An ntroduction using r,* Chichester, England, John Wiley & Sons, Ltd.

Croshaw, D. & Pechmann, J. 2015. Size does not matter for male marbled salamanders (ambystoma opacum). *Canadian Journal of Zoology,* 93**,** 735-740.

Daly, M. 1978. The cost of mating. *American Naturalist*, 112, 771-774.

Danielsson, I. 1998. Mechanisms of sperm competition in insects. *Annales Zoologici Fennici,* 35**,** 241-257.

Darwin, C. 1871. *The descent of man and selection in relation to sex,* London, John Murray.

Dawkins, M. S. & Guilford, T. 1991. The corruption of honest signalling. *Animal Behaviour,* 41**,** 865-873.

De Boer, B. A. 1981. Influence of population density on the territorial, courting and spawning behaviour of male chromis cyanea (pomacentridae). *Behaviour,* 77**,** 99-120.

Dibattista, J.; Feldheim, K.; Gruber, S. & Hendry, A. 2007. When bigger is not better: Selection against large size, high condition and fast growth in juvenile lemon sharks. *Journal of Evolutionary Biology,* 20**,** 201-212.

Dingemanse, N. J.; Kazem, A. J.; Réale, D. & Wright, J. 2010. Behavioural reaction norms: Animal personality meets individual plasticity. *Trends in Ecology & Evolution,* 25**,** 81-89.

Dirienzo, N. & Marshall, J. L. 2013. Function of the hemolymph nuptial gift in the ground cricket, allonemobius socius. *Ethology,* 119**,** 104-109.

Dolgin, E. S.; Whitlock, M. C. & Agrawal, A. F. 2006. Male drosophila melanogaster have higher mating success when adapted to their thermal environment. *Journal of Evolutionary Biology,* 19**,** 1894-1900.

Doutrelant, C. & Mcgregor, P. K. 2000. Eavesdropping and mate choice in female fighting fish. *Behaviour,* 137**,** 1655-1668.

Drayton, J. M.; Boeke, J. K. & Jennions, M. D. 2013. Immune challenge and pre-and post-copulatory female choice in the cricket teleogryllus commodus. *Journal of insect behavior,* 26**,** 176-190.

Drickamer, L. C.; Gowaty, P. A. & Holmes, C. M. 2000. Free female mate choice in house mice affects reproductive success and offspring viability and performance. *Animal Behaviour,* 59**,** 371-378.

Edward, D. A. 2014. The description of mate choice. *Behavioral Ecology,* 26**,** 301-310.

Elias, D. O.; Kasumovic, M. M.; Punzalan, D.; Andrade, M. C. B. & Mason, A. C. 2008. Assessment during aggressive contests between male jumping spiders. *Animal Behaviour,* 76**,** 901-910.

Emlen, S. T. & Oring, L. W. 1977. Ecology, sexual slection and the evolution of mating systems. *Science,* 197**,** 215-223.

Endler, J. A. & Houde, A. E. 1995. Geographic variation in female preferences for male traits in poecilia reticulata. *Evolution,* 49**,** 456-468.

Fan, X.-L.; Lin, Z.-H. & Ji, X. 2013. Male size does not correlate with fertilization success in two bufonid toads that show size-assortative mating. *Current Zoology,* 59**,** 740-746.

Fedorka, K. M. & Mousseau, T. A. 2002. Tibial spur feeding in ground crickets: Larger males contribute larger gifts (orthoptera : Gryllidae). *Florida Entomologist,* 85**,** 317-323.

Fedorka, K. M. & Mousseau, T. A. 2007. Immune system activation affects male sexual signal and reproductive potential in crickets. *Behavioral Ecology,* 18**,** 231-235.

Fedorka, K. M.; Zuk, M. & Mousseau, T. A. 2004. Immune suppression and the cost of reproduction in the ground cricket, allonemobius socius. *Evolution,* 58**,** 2478-2485.

Filippi-Codaccioni, O.; Moussus, J. P.; Urcun, J. P. & Jiguet, F. 2010. Advanced departure dates in long-distance migratory raptors. *Journal of Ornithology,* 151**,** 687-694.

Firman, R. C. & Simmons, L. W. 2012. Male house mice evolving with post-copulatory sexual selection sire embryos with increased viability. *Ecology Letters,* 15**,** 42-46.

Fisher, D. N.; James, A.; Rodríguez-Muñoz, R. & Tregenza, T. 2015. Behaviour in captivity predicts some aspects of natural behaviour, but not others, in a wild cricket population. *Proceedings of the Royal Society B: Biological Sciences,* 282**,** 20150708.

Fitzsimmons, L. P. & Bertram, S. M. 2011. The calling songs of male spring field crickets (gryllus veletis) change as males age. *Behaviour,* 148**,** 1045-1065.

Fitzsimmons, L. P. & Bertram, S. M. 2013. No relationship between long-distance acoustic mate attraction signals and male fertility or female preference in spring field crickets. *Behavioral ecology and sociobiology,* 67**,** 885-893.

Fleischman, R. R. & Sakaluk, S. K. 2004. No direct or indirect benefits to cryptic female choice in house crickets (acheta domesticus). *Behavioral Ecology,* 15**,** 793-798.

Forrest, T. G.; Sylvester, J. L.; Testa, S.; Smith, S. W.; Dinep, A.; Cupit, T. L.; Huggins, J. M.; Atkins, K. L. & Eubanks, M. 1991. Mate choice in ground crickets (gryllidae, nemoiinae). *Florida Entomologist,* 74**,** 74-80.

Forsman, A. & Hagman, M. 2006. Calling is an honest indicator of paternal genetic quality in poison frogs. *Evolution,* 60**,** 2148-2157.

Fraser, D. F. & Gilliam, J. F. 1992. Nonlethal impacts of predator invasion: Facultative suppression of growth and reproduction. *Ecology,* 73**,** 959-970.

Fricke, C. & Arnqvist, G. 2007. Rapid adaptation to a novel host in a seed beetle (callosobruchus maculatus): The role of sexual selection. *Evolution,* 61**,** 440-454.

Gabor, C.R. & Halliday, T.R. 1997. Sequential mate choice by multiply mating smooth newts: females become more choosy. *Behavioural ecology*, 8, 162-166.

Gage, M.J.G. 1995. Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc R Soc B*, 261, 25-30.

Gayon, J. 2010. Sexual selection: Another darwinian process. *Comptes rendus biologies,* 333**,** 134-144.

Gershman, S. N. & Sakaluk, S. K. 2010. Mate quality and novelty influence post-copulatory female choice in decorated crickets, gryllodes sigillatus. *Ethology,* 116**,** 1113-1117.

Gibson, J.S. & Uetz, G.W. 2008. Seismic communication and mate choice in wolf spiders: components of male seismic signals and mating success. *Animal Behaviour*, 75, 1253-1262.

Godin, J.J. & McDonough, H.E. 2003, Predatory preference for brightly colored males in the guppy: a viability cost for a sexually selected trait. *Behavioural Ecology*, 14, 194-200.

Grafen, A. 1990a. Biological signals as handicaps. *Journal of Theoretical Biology,* 144**,** 517-546.

Grafen, A. 1990b. Sexual selection unhandicapped by the fisher process. *Journal of Theoretical Biology,* 144**,** 473-516.

Gray, B. & Simmons, L. W. 2013. Acoustic cues alter perceived sperm competition risk in the field cricket teleogryllus oceanicus. *Behavioral Ecology,* 24**,** 982-986.

Gray, D. A. 1997. Female house crickets, acheta domesticus, prefer the chirps of large males. *Animal behaviour,* 54**,** 1553-1562.

Gray, D. A. 1999. Intrinsic factors affecting female choice in house crickets: Time cost, female age, nutritional condition, body size, and size-relative reproductive investment. *Journal of Insect Behavior,* 12**,** 691-700.

Gray, D. A. & Eckhardt, G. 2001. Is cricket courtship song condition dependent? *Animal Behaviour,* 62**,** 871-877.

Grazer, V. M.; Demont, M.; Michalczyk, L.; Gage, M. J. G. & Martin, O. Y. 2014. Environmental quality alters female costs and benefits of evolving under enforced monogamy. *BMC Evolutionary Biology,* 14**,** 1-7.

Green, D. M. 2017. Amphibian breeding phenology trends under climate change: Predicting the past to forecast the future. *Global Change Biology,* 23**,** 646-656.

Green, K. 2008. *Acoustic signalling and mate choice in field crickets.* Doctor of Philosophy in Biological Sciences, University of Exeter.

Green, K. & Tregenza, T. 2009. The influence of male ejaculates on female mate search behaviour, oviposition and longevity in crickets. *Animal Behaviour,* 77**,** 887-892.

Greenfield, M. D. & Shelly, T. E. 1985. Alternative mating strategies in a desert grasshopper: Evidence of density-dependence. *Animal Behaviour,* 33**,** 1192-1210.

Grieshop, K.; Stangberg, J.; Martinossi-Allibert, I.; Arnqvist, G. & Berger, D. 2016. Strong sexual selection in males against a mutation load that reduces offspring production in seed beetles. *Journal of Evolutionary Biology,* 29**,** 1201-1210.

Gross, M.T.; 1996. Alternative reproductive strategies and tactics: diversity within sexes. *Trends in ecology and evolution*, 11, 92-98.

Hack, M. A. 1998. The energetics of male mating strategies in field crickets (orthoptera : Gryllinae : Gryllidae). *Journal of Insect Behavior,* 11**,** 853-867.

Haldane, J. B. S. 1932. *The causes of evolution,* Ithaca, NY, Cornell University Press.

Hamilton, W. D. & Zuk, M. 1982. Heritable true fitness and bright birds - a role for parasites. *Science,* 218**,** 384-387.

Harrison, J. F.; Cease, A. J.; Vandenbrooks, J. M.; Albert, T. & Davidowitz, G. 2013a. Caterpillars selected for large body size and short development

time are more susceptible to oxygen-related stress. *Ecology and evolution,* 3**,** 1305-1316.

Harrison, S. J.; Thomson, I. R.; Grant, C. M. & Bertram, S. M. 2013b. Calling, courtship, and condition in the fall field cricket, gryllus pennsylvanicus. *PloS one,* 8**,** e60356.

Harrison, X. A. 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ,* 2**,** 1-19.

Harrison, X. A. 2015. A comparison of observation-level random effect and beta-binomial models for modelling overdispersion in binomial data in ecology & evolution. *PeerJ,* 3**,** 1-17.

Hebets, E. A.; Wesson, J. & Shamble, P. S. 2008. Diet influences mate choice selectivity in adult female wolf spiders. *Animal Behaviour,* 76**,** 355-363.

Hedrick, A. V. 1986. Female preferences for male calling bout duration in a field cricket. *Behavioral Ecology and Sociobiology,* 19**,** 73-77.

Hill, G.E. 1990. Female house finches prefer colourful males: sexual selection for a condition-dependent trait. *Animal Behaviour*, 40, 563-572

Hill, G. E. 1991. Plumage colorations is a sexually selected indicator of male quality. *Nature,* 350**,** 337-339.

Hissmann, K. 1990. Strategies of mate finding in the european field cricket (gryllus campestris) at different population densities: A field study. *Ecological Entomology,* 15**,** 281-291.

Hoback, W. W. & Wagner, W. E. 1997. The energetic cost of calling in the variable field cricket, gryllus lineaticeps. *Physiological Entomology,* 22**,** 286-290.

Hochkirch, A.; Witzenberger, K. A.; Teerling, A. & Niemeyer, F. 2007. Translocation of an endangered insect species, the field cricket (gryllus

campestris linnaeus, 1758) in northern germany. *Biodiversity and Conservation,* 16**,** 3597-3607.

Hockham, L. R. & Vahed, K. 1997. The function of mate guarding in a field cricket (orthoptera: Gryllidae; teleogryllus natalensis otte and cade). *Journal of Insect Behavior,* 10**,** 247-256.

Höjesjö, J.; Johnsson, J. I. & Bohlin, T. 2002. Can laboratory studies on dominance predict fitness of young brown trout in the wild? *Behavioral Ecology and Sociobiology,* 52**,** 102-108.

Holland, B. 2002. Sexual selection fails to promote adaptation to a new environment. *Evolution,* 56**,** 721-730.

Holland, B. & Rice, W. R. 1998. Perspective: Chase-away sexual selection: Antagonistic seduction versus resistance. *Evolution,* 52**,** 1-7.

Holland, B. & Rice, W. R. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences,* 96**,** 5083-5088.

Hollis, B.; Fierst, J. L. & Houle, D. 2009. Sexual selection accelerates the elimination of a deleterious mutant in drosophila melanogaster. *Evolution,* 63**,** 324-333.

Hollis, B. & Houle, D. 2011. Populations with elevated mutation load do not benefit from the operation of sexual selection. *Journal of Evolutionary Biology,* 24**,** 1918-1926.

Hollis, B. & Kawecki, T. J. 2014. Male cognitive performance declines in the absence of sexual selection. *Proceedings of the Royal Society B-Biological Sciences,* 281**,** 1-7.

Holzer, B.; Jacot, A. & Brinkhof, M. W. G. 2003. Condition-dependent signaling affects male sexual attractiveness in field crickets, gryllus campestris. *Behavioral Ecology,* 14**,** 353-359.

Hopwood, P.E.; Moore, A.J.; Royle, N.J. 2013. Nutrition during sexual maturation affects competitive ability but not reproductive productivity in burying beetles. *Functional ecology*, 27, 1350-1357.

Hopwood, P.E.; Moore, A.J.; Royle, N.J. 2014. Effects of resource variation during early life and adult social environment in contest outcomes in burying beetles: a context-dependent silver spoon strategy?. *Proc R Soc Lond B*, 281, 20133102.

Hosken, D. J. & House, C. M. 2011. Sexual selection. *Current Biology,* 21**,** R62-R65.

Houle, D. 1991. Genetic covariance of fitness correlates - what genetic correlations are made of and why it matters. *Evolution,* 45**,** 630-648.

Hsu, Y. & Wolf, L. L. 1999. The winner and loser effect: Integrating multiple experiences. *Animal Behaviour,* 57**,** 903-910.

Hughes, K. A.; Houde, A. E.; Price, A. C. & Rodd, F. H. 2013. Mating advantage for rare males in wild guppy populations. *Nature,* 503**,** 108-110.

Hunt, J.; Brooks, R. & Jennions, Michael d. 2005. Female mate choice as a condition-dependent life-history trait. *The American Naturalist,* 166**,** 79-92.

Hunt, J.; Brooks, R.; Jennions, M. D.; Smith, M. J.; Bentsen, C. L. & Bussiere, L. F. 2004. High-quality male field crickets invest heavily in sexual display but die young. *Nature,* 432**,** 1024-1027.

Hunt, S.; Cuthill, I. C.; Bennett, A. T. & Griffiths, R. 1999. Preferences for ultraviolet partners in the blue tit. *Animal Behaviour,* 58**,** 809-815.

Iglesias-Carrasco, M.; Head, M. L.; Jennions, M. D. & Cabido, C. 2017. Secondary compounds from exotic tree plantations change female mating preferences in the palmate newt (lissotriton helveticus). *Journal of Evolutionary Biology,* 30**,** 1788-1795.

Immonen, E.; Hoikkala, A.; Kazem, A. J. & Ritchie, M. G. 2009. When are vomiting males attractive? Sexual selection on condition-dependent nuptial feeding in drosophila subobscura. *Behavioral Ecology,* 20**,** 289-295.

Ismail, M.; Vernon, P.; Hance, T.; Pierre, J. S. & Van Baaren, J. 2012. What are the possible benefits of small size for energy-constrained ectotherms in cold stress conditions? *Oikos,* 121**,** 2072-2080.

Ivy, T. M. & Sakaluk, S. K. 2007. Sequential mate choice in decorated crickets: Females use a fixed internal threshold in pre-and postcopulatory choice. *Animal Behaviour,* 74**,** 1065-1072.

Jacomb, F.; Marsh, J. & Holman, L. 2016. Sexual selection expedites the evolution of pesticide resistance. *Evolution,* 70**,** 2746-2751.

Jacot, A.; Scheuber, H. & Brinkhof, M. W. G. 2007. The effect of age on a sexually selected acoustic display. *Ethology,* 113**,** 615-620.

Jacot, A.; Scheuber, H.; Holzer, B.; Otti, O. & Brinkhof, M. W. G. 2008. Diel variation in a dynamic sexual display and its association with female mate-searching behaviour. *Proceedings of the Royal Society B: Biological Sciences,* 275**,** 579-585.

Jacot, A.; Scheuber, H.; Kurtz, J. & Brinkhof, M. 2005. Juvenile immune status affects the expression of a sexually selected trait in field crickets. *Journal of evolutionary biology,* 18**,** 1060-1068.

Jarzebowska, M. & Radwan, J. 2010. Sexual selection counteracts extinction of small populations of the bulb mites. *Evolution,* 64**,** 1283-1289.

Jennions, M.; Drayton, J.; Brooks, R. & Hunt, J. 2007. Do female black field crickets teleogryllus commodus benefit from polyandry? *Journal of evolutionary biology,* 20**,** 1469-1477.

Jennions, M. D. & Petrie, M. 1997. Variation in mate choice and mating preferences: A review of causes and consequences. *Biological Reviews,* 72**,** 283-327.

Jirotkul, M. 1999a. Population density influences male–male competition in guppies. *Animal Behaviour,* 58**,** 1169-1175.

Jirotkul, M. 1999b. Operational sex ratio influences female preference and male-male competition in guppies. *Animal Behaviour*, 58, 287-294.

Johnson, S. & Candolin, U. 2017. Predation cost of a sexual signal in the threespine stickleback. *Behavioural Ecology*, 28, 1160-1165.

Johnstone, R. A. & Grafen, A. 1993. Dishonesty and the handicap principle. *Animal Behaviour,* 46**,** 759-764.

Jones, A. G.; Arguello, J. R. & Arnold, S. J. 2002. Validation of bateman's principles: A genetic study of sexual selection and mating patterns in the rough–skinned newt. *Proceedings of the Royal Society of London B: Biological Sciences,* 269**,** 2533-2539.

Jones, A. G.; Arguello, J. R. & Arnold, S. J. 2004. Molecular parentage analysis in experimental newt populations: The response of mating system measures to variation in the operational sex ratio. *The American Naturalist,* 164**,** 444-456.

Jönsson, P. E. & Alerstam, T. 1990. The adaptive significance of parental role division and sexual size dimorphism in breeding shorebirds. *Biological Journal of the Linnean Society,* 41**,** 301-314.

Judge, K. A. 2010. Female social experience affects the shape of sexual selection on males. *Evolutionary Ecology Research,* 12**,** 389-402.

Judge, K. A. 2011. Do male field crickets, gryllus pennsylvanicus, signal their age? *Animal behaviour,* 81**,** 185-194.

Judge, K. A.; Ting, J. J. & Gwynne, D. T. 2008. Condition dependence of male life span and calling effort in a field cricket. *Evolution,* 62**,** 868-878.

Judge, K. A.; Ting, J. J. & Gwynne, D. T. 2014. Condition dependence of female choosiness in a field cricket. *Journal of Evolutionary Biology,* 27**,** 2529-2540.

Judge, K. A.; Tran, K. C. & Gwynne, D. T. 2010. The relative effects of mating status and age on the mating behaviour of female field crickets. *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 88**,** 219-223.

Kelly, C. D.; Telemeco, M. S. & Bartholomay, L. C. 2015. Are attractive male crickets better able to pay the costs of an immune challenge? *PeerJ,* 3**,** e1501.

Ketola, T.; Kortet, R. & Kotiaho, J. S. 2007. Testing theories of sexual selection in decorated crickets (gryllodes sigillatus). *Evolutionary Ecology Research,* 9**,** 869-885.

Ketola, T.; Kortet, R. & Kotiaho, J. S. 2009. Endurance in exercise is associated with courtship call rate in decorated crickets, gryllodes sigillatus. *Evolutionary Ecology Research,* 11**,** 1131-1139.

Keyser, A. J. & Hill, G. E. 2000. Structurally based plumage coloration is an honest signal of quality in male blue grosbeaks. *Behavioral Ecology,* 11**,** 202-209.

Kindle, T. K.; Johnson, K. M.; Ivy, T. M.; Weddle, C. B. & Sakaluk, S. K. 2006. Female mating frequency increases with temperature in two cricket species, gryllodes sigillatus and acheta domesticus (orthoptera: Gryllidae). *Canadian journal of zoology,* 84**,** 1345-1350.

Kleinteich, A; Wilder S.M.; Schneider J.M. 2015, Contributions of juvenile and adult diet to the lifetime reproductive success and lifespan of a spider. *Oikos*, 124, 130-138.

Knapp, R. A. & Kovach, J. T. 1991. Courtship as an honest indicator of male parental quality in the bicolor damselfish, stegastes partitus. *Behavioral Ecology,* 2**,** 295-300.

Kodric-Brown, A. & Nicoletto, P.F. 2000, Age and experience affect female choice in the Guppy (*Poecilia reticulata*). *American Naturalist*, 157, 316-323.

Kokko, H. & Brooks, R. 2003. Sexy to die for? Sexual selection and the risk of extinction. *Ann. Zool. Fenn.,* 40**,** 207-219.

Kokko, H.; Brooks, R.; Jennions, M.D.; Morley, J. 2003. The evolution of mate choice and mating biases. *Proc. R. Soc. Lond. B*, 270, 653-664.

Kokko, H. & Mappes, J. 2005. Sexual selection when fertilization is not guaranteed. *Evolution,* 59**,** 1876-1885.

Kortet, R. & Hedrick, A. 2005. The scent of dominance: Female field crickets use odour to predict the outcome of male competition. *Behavioral Ecology and Sociobiology,* 59**,** 77-83.

Kovach, R. P.; Joyce, J. E.; Echave, J. D.; Lindberg, M. S. & Tallmon, D. A. 2013. Earlier migration timing, decreasing phenotypic variation, and biocomplexity in multiple salmonid species. *Plos One,* 8**,** 1-10.

Kuczynski, M. C.; Getty, T. & Gering, E. 2017. Larger females are choosier in the gray treefrog (hyla versicolor). *Behavioural Processes,* 135**,** 29-35.

Kuriwada, T. 2016. Horn length is not correlated with calling efforts in the horn-headed cricket loxoblemmus doenitzi (orthoptera: Gryllidae). *Entomological Science,* 19**,** 228-232.

Kuriwada, T. & Kasuya, E. 2006. Female responsiveness is negatively correlated with life expectancy in meloimorpha japonica (orthoptera : Gryllidae). *Annals of the Entomological Society of America,* 99**,** 1244-1247.

Kuriwada, T. & Kasuya, E. 2011. Age-dependent changes in calling effort in the bell cricket meloimorpha japonica. *Journal of ethology,* 29**,** 99-105.

Lailvaux, S. P. & Irschick, D. J. 2006. A functional perspective on sexual selection: Insights and future prospects. *Animal Behaviour,* 72**,** 263-273.

Lamunyon, C. W. & Ward, S. 1998. Larger sperm outcompete smaller sperm in the nematode caenorhabditis elegans. *Proceedings of the Royal Society B-Biological Sciences,* 265**,** 1997-2002.

Lande, R. 1976. Natural selection and random genetic drift in phenotypic evolution. *Evolution,* 30**,** 314-334.

Lappin, A. K. & Husak, J. F. 2005. Weapon performance, not size, determines mating success and potential reproductive output in the collared lizard (crotaphytus collaris). *The American Naturalist,* 166**,** 426-436.

Lee, Y. & Nelder, J. A. 1996. Hierarchical generalized linear models. *Journal of the Royal Statistical Society Series B-Methodological,* 58**,** 619-656.

Leonard, A. S. & Hedrick, A. V. 2010. Long-distance signals influence assessment of close range mating displays in the field cricket, gryllus integer. *Biological Journal of the Linnean Society,* 100**,** 856-865.

Lickman, K.; Murray, A. M. & Cade, W. H. 1998. Effect of mating on female phonotactic response in gryllus integer (orthoptera : Gryllidae). *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 76**,** 1263-1268.

Lierheimer, V. F. & Tinghitella, R. M. 2017. Quantity and quality of available mates alters female responsiveness but not investment in the pacific field cricket, teleogryllus oceanicus. *Behavioral Ecology and Sociobiology,* 71**,** 80-90.

Lindström, K. & Lehtonen, T. K. 2013. Mate sampling and choosiness in the sand goby. *Proceedings of the Royal Society of London B: Biological Sciences,* 280**,** 20130983.

Lindström, K. & Lundström, J. 2000. Male greenfinches (*Carduelis chloris*) with brighter ornaments have higher virus infection clearance rate. *Behavioral Ecology and Sociobiology*, 48, 44–51.

Locatello, L.; Mazzoldi, C.; Santon, M.; Sparaciari, S. & Rasotto, M. 2016. Unexpected female preference for smaller males in the marbled goby pomatoschistus marmoratus. *Journal of fish biology,* 89**,** 1845-1850.

Loher, W. & Dambach, M. 1989. Reproductive behaviour. *In:* HUBER, F., MOORE, T. E. & LOHER, W. (eds.) *Cricket behaviour and neurobiology.* Ithaca, NY: Cornell University Press.

Long, Tristan a. F.; Agrawal, Aneil f. & Rowe, L. 2012. The effect of sexual selection on offspring fitness depends on the nature of genetic variation. *Current Biology,* 22**,** 204-208.

Loranger, M. J. & Bertram, S. M. 2016. The effect of male dominance on female choice in a field cricket (gryllus assimilis). *Animal Behaviour,* 114**,** 45-52.

Lorch, P. D.; Proulx, S.; Rowe, L. & Day, T. 2003. Condition-dependent sexual selection can accelerate adaptation. *Evolutionary Ecology Research,* 5**,** 867-881.

Lumley, A. J.; Michalczyk, L.; Kitson, J. J. N.; Spurgin, L. G.; Morrison, C. A.; Godwin, J. L.; Dickinson, M. E.; Martin, O. Y.; Emerson, B. C.; Chapman, T. & Gage, M. J. G. 2015. Sexual selection protects against extinction. *Nature,* 522**,** 470-473.

Macario, A.; Croft, D. P.; Endler, J. A. & Darden, S. K. 2017. Early social experience shapes female mate choice in guppies. *Behavioral Ecology,* 28**,** 833-843.

Maclellan, K.; Whitlock, M. C. & Rundle, H. D. 2009. Sexual selection against deleterious mutations via variable male search success. *Biology Letters,* 5**,** 795-797.

Maddocks, S. A.; Bennett, A. T.; Hunt, S. & Cuthill, I. C. 2002. Context-dependent visual preferences in starlings and blue tits: Mate choice and light environment. *Animal behaviour,* 63**,** 69-75.

Marshall, J. A. & Haes, E. C. M. 1988. *Grasshoppers and allied insects of great britain and ireland*, Harley Books.

Martin, O. Y.; Hosken, D. J. & Ward, P. I. 2004. Post–copulatory sexual selection and female fitness in scathophaga stercoraria. *Proceedings of the Royal Society of London B: Biological Sciences,* 271**,** 353-359.

Martin, S. D.; Gray, D. A. & Cade, W. H. 2000. Fine-scale temperature effects on cricket calling song. *Canadian Journal of Zoology,* 78**,** 706-712.

Mason, L. 1964. Stabilizing selection for mating fitness in natural populations of tetraopes. *Evolution,* 18**,** 492-497.

Mautz, B. S. & Sakaluk, S. K. 2008. The effects of age and previous mating experience on pre- and post-copulatory mate choice in female house crickets (acheta domesticus l.). *Journal of Insect Behavior,* 21**,** 203-212.

Maynard Smith, J. & Harper, D. 1988. The evolution of aggression: Can selection generate variability? *Philisophical Transactions of the Royal Society of London Series B,* 319**,** 557-570.

Mcauley, E. M. & Bertram, S. M. 2016. Field crickets compensate for unattractive static long-distance call components by increasing dynamic signalling effort. *PloS one,* 11**,** e0167311.

Milanović, S.; Janković-Tomanić, M.; Kostić, I.; Kostić, M.; Morina, F.; Živanović, B. & Lazarević, J. 2016. Behavioural and physiological plasticity of gypsy moth larvae to host plant switching. *Entomologia Experimentalis et Applicata,* 158**,** 152-162.

Mineka, S.; Keir, R. & Price, V. 1980. Fear of snakes in wild-and laboratory-reared rhesus monkeys (macaca mulatta). *Learning & Behavior,* 8**,** 653-663.

Molina-Borja, M.; Padron-Fumero, M. & Alfonso-Martin, T. 1998. Morphological and behavioural traits affecting the intensity and outcome of male contests in gallotia galloti galloti (family lacertidae). *Ethology,* 104**,** 314-322.

Møller, A. P. & Alatalo, R.V. 1999. Good-genes effects in sexual selection. *Proc. R. Soc. Lond. B*, 266, 85-91.

Møller, A. P. & Pomiankowski, A. 1993. Why have birds got multiple sexual ornaments. *Behavioral Ecology and Sociobiology,* 32**,** 167-176.

Moore, A. J. 1990. The evolution of sexual dimorphism by sexual selection: The separate effects of intrasexual selection and intersexual selection. *Evolution,* 44**,** 315-331.

Moore, P. J. & Moore, A. J. 2001. Reproductive aging and mating: The ticking of the biological clock in female cockroaches. *Proceedings of the National Academy of Sciences,* 98**,** 9171-9176.

Mougeot, F & Bretagnolle, V. 2000. Predation as a cost of sexual communication in nocturnal seabirds: an experimental approach using acoustic signals. *Animal behaviour*, 60, 647-656.

Mowles, S. L. 2014. The physiological cost of courtship: Field cricket song results in anaerobic metabolism. *Animal Behaviour,* 89**,** 39-43.

Mowles, S.L.; Cotton, P.A.; Briffa, M. 2009. Aerobic capacity influences giving-up decisions in fighting hermit crabs: does stamina constrain contests?. *Animal Behaviour*, 78, 735-740.

Mowles, S. L.; Cotton, P. A. & Briffa, M. 2010. Whole-organism performance capacity predicts resource-holding potential in the hermit crab pagurus bernhardus. *Animal Behaviour,* 80**,** 277-282.

Mowles, S. L.; Cotton, P. A. & Briffa, M. 2011. Flexing the abdominals: Do bigger muscles make better fighters? *Biology Letters,* 7**,** 358-360.

Neems, R. M.; Lazarus, J. & Mcadllan, A. J. 1998. Lifetime reproductive success in a swarming midge: Trade-offs and stabilizing selection for male body size. *Behavioral Ecology,* 9**,** 279-286.

Nelissen, M. H. J. 1992. Does body size affect the ranking of a cichlid fish in a dominance hierarchy? *Journal of Ethology,* 10**,** 153-156.

Nelson, C. M. & Nolen, T. G. 1997. Courtship song, male agonistic encounters, and female mate choice in the house cricket, acheta domesticus (orthoptera: Gryllidae). *Journal of Insect Behavior,* 10**,** 557-570.

Nijhout, H. F. 1979. Stretch-induced molting in oncopeltus fasciatus. *Journal of Insect Physiology,* 25**,** 277-281.

Nijhout, H. F. & Williams, C. M. 1974. Control of molting and metamorphosis in tobacco hornworm, manduca sexta (l) - growth of last-instar larva and decision to pupate. *Journal of Experimental Biology,* 61**,** 481-491.

Olvido, A. E. & Wagner, W. E. 2004. Signal components, acoustic preference functions and sexual selection in a cricket. *Biological Journal of the Linnean Society,* 83**,** 461-472.

Ophir, A.; Schrader, S. & Gillooly, J. 2010. Energetic cost of calling: General constraints and species-specific differences. *Journal of evolutionary biology,* 23**,** 1564-1569.

Ophir, A. G. & Galef, B. G. 2003. Female japanese quail that 'eavesdrop'on fighting males prefer losers to winners. *Animal Behaviour,* 66**,** 399-407.

Pacheco, K. & Bertram, S. M. 2014. How male sound pressure level influences phonotaxis in virgin female jamaican field crickets (gryllus assimilis). *PeerJ,* 2**,** e437.

Pacheco, K.; Dawson, J. W.; Jutting, M. & Bertram, S. M. 2013. How age influences phonotaxis in virgin female jamaican field crickets (gryllus assimilis). *PeerJ,* 1**,** 1-17.

Parker, G. 1979. Sexual selection and sexual conflict. *In:* BLUM, M. & BLUM, N. (eds.) *Sexual selection and reproductive competition in insects.* New York, NY: Academic Press Inc.

Parker, G. A. 1974. Assessment strategy and evolution of fighting behaviour. *Journal of Theoretical Biology,* 47**,** 223-243.

Parker, G. A.; Baker, R. & Smith, V. 1972. The origin and evolution of gamete dimorphism and the male-female phenomenon. *Journal of theoretical biology,* 36**,** 529-553.

Part, T. & Qvarnstrom, A. 1997. Badge size in collared flycatchers predicts outcome of male competition over territories. *Animal Behaviour,* 54**,** 893-899.

Partridge, L. 1980. Mate choice increases a component of offspring fitness in fruit-flies. *Nature,* 283**,** 290-291.

Passow, C. N.; Greenway, R.; Arias-Rodriguez, L.; Jeyasingh, P. D. & Tobler, M. 2015. Reduction of energetic demands through modification of body size and routine metabolic rates in extremophile fish. *Physiological and Biochemical Zoology,* 88**,** 371-383.

Patterson, B. D.; Kays, R. W.; Kasiki, S. M. & Sebestyen, V. M. 2006. Developmental effects of climate on the lion's mane (panthera leo). *Journal of Mammalogy,* 87**,** 193-200.

Patton, R. L. 1978. Growth and development parameters of acheta domesticus (orthoptera, gryllidae). *Annals of the Entomological Society of America,* 71**,** 40-42.

Pearce-Kelly, P.; Jones, R.; Clarke, D.; Walker, C.; Atkin, P. & Cunningham, A. 1998. The captive rearing of threatened orthoptera: A comparison of the conservation potential and practical considerations of two species' breeding programmes at the zoological society of london. *Journal of Insect Conservation,* 2**,** 201-210.

Peig, J. & Green, A. J. 2009. New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos,* 118**,** 1883-1891.

Petrie, M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature,* 371**,** 598-599.

Petrie, M. & Halliday, T. 1994. Experimental and natural changes in the peacock's (pavo cristatus) train can affect mating success. *Behavioral Ecology and Sociobiology,* 35**,** 213-217.

Petrie, M.; Tim, H. & Carolyn, S. 1991. Peahens prefer peacocks with elaborate trains. *Animal Behaviour,* 41**,** 323-331.

Pfennig, K. S.; Pfennig, D. W.; Porter, C. & Martin, R. A. 2015. Sexual selection's impacts on ecological specialization: An experimental test. *Proceedings of the Royal Society B-Biological Sciences,* 282**,** 1-8.

Pitcher, T.E.; Neff, B.D.; Rodd, F.H.; Rowe,L. 2003. Multiple mating and sequential mate choice in guppies: females trade up. *Proc. R. Soc. Lond. B*, 270, 1623-1629

Plesnar, A.; Konior, M. & Radwan, J. 2011. The role of sexual selection in purging the genome of induced mutations in the bulb mite (rizoglyphus robini). *Evolutionary Ecology Research,* 13**,** 209-216.

Plesnar-Bielak, A.; Woch, K.R.; Malszycki, M.A.; Alkhawlany, A.T.H.; Holysz, A.; Assis Correia, J.F.; Turk, N.; Ugrin, M.; Kramarz, P.; Prokop, Z.M. 2017. Larval and adult nutrition effects on reproductive traits in the red flour beetle. *Journal of Zoology*, 302, 79-87.

Poloczanska, E. S.; Brown, C. J.; Sydeman, W. J.; Kiessling, W.; Schoeman, D. S.; Moore, P. J.; Brander, K.; Bruno, J. F.; Buckley, L. B.; Burrows, M. T.; Duarte, C. M.; Halpern, B. S.; Holding, J.; Kappel, C. V.; O'connor, M. I.;

Pandolfi, J. M.; Parmesan, C.; Schwing, F.; Thompson, S. A. & Richardson, A. J. 2013. Global imprint of climate change on marine life. *Nature Climate Change,* 3**,** 919-925.

Pomiankowski, A. 1987. The costs of choice in sexual selection. *Journal of Theoretical Biology,* 128**,** 195-218.

Ponce-Wainer, J. X. & Del Castillo, R. C. 2008. Female mate choice and no detected predation risk in relation to the calling song of oecanthus nivelus (gryllidae : Oecanthinae). *Annals of the Entomological Society of America,* 101**,** 260-265.

Popov, A. & Shuvalov, V. 1977. Phonotactic behavior of crickets. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology,* 119**,** 111-126.

Power, D. J. & Holman, L. 2015. Assessing the alignment of sexual and natural selection using radiomutagenized seed beetles. *Journal of Evolutionary Biology,* 28**,** 1039-1048.

Price, T. & Schluter, D. 1991. On the low heritability of life-history traits. *Evolution,* 45**,** 853-861.

Prokop, P. & Maxwell, M. R. 2008. Interactions between multiple forms of nuptial feeding in the wood cricket nemobius sylvestris (bosc): Dual spermatophores and male forewings. *Ethology,* 114**,** 1173-1182.

Promislow, D. E. L.; Smith, E. A. & Pearse, L. 1998. Adult fitness consequences of sexual selection in drosophila melanogaster. *Proceedings of the National Academy of Sciences of the United States of America,* 95**,** 10687-10692.

Prosser, M. R.; Murray, A. M. & Cade, W. H. 1997. The influence of female age on phonotaxis during single and multiple song presentations in the field

cricket, gryllus integer (orthoptera: Gryllidae). *Journal of Insect Behavior,* 10**,** 437-449.

Qvarnstrom, A. & Forsgren, E. 1998. Should females prefer dominant males? *Trends in Ecology & Evolution,* 13**,** 498-501.

Qvarnstrom, A.; Part, T. & Sheldon, B. C. 2000. Adaptive plasticity in mate preference linked to differences in reproductive effort. *Nature,* 405**,** 344-347.

Radwan, J. 1996. Intraspecific variation in sperm competition success in the bulb mite: A role for sperm size. *Proceedings of the Royal Society B-Biological Sciences,* 263**,** 855-859.

Radwan, J. 2004. Effectiveness of sexual selection in removing mutations induced with ionizing radiation. *Ecology Letters,* 7**,** 1149-1154.

Radwan, J.; Unrug, J.; Śnigórska, K. & Gawrońska, K. 2004. Effectiveness of sexual selection in preventing fitness deterioration in bulb mite populations under relaxed natural selection. *Journal of Evolutionary Biology,* 17**,** 94-99.

Rantala, M. J. & Kortet, R. 2003. Courtship song and immune function in the field cricket gryllus bimaculatus. *Biological Journal of the Linnean Society,* 79**,** 503-510.

Rapkin, J.; Archer, R. C.; Grant, C. E.; Jensen, K.; House, C. M.; Wilson, A. J. & Hunt, J. 2017. Little evidence for intralocus sexual conflict over the optimal intake of nutrients for lifespan and reproduction in the black field cricket teleogryllus commodus. *Evolution***,** 2159-2177.

Rapkin, J.; Jensen, K.; Archer, C. R.; House, C. M.; Sakaluk, S. K.; Castillo, E. D. & Hunt, J. 2018. The geometry of nutrient space–based life-history trade-offs: Sex-specific effects of macronutrient intake on the trade-off

between encapsulation ability and reproductive effort in decorated crickets. *The American Naturalist,* 191**,** 452-474.

Rapkin, J.; Jensen, K.; House, C.M.; Sakaluk, S.K.; Sakaluk, J.K.; Hunt, J. 2016. The complex interplay between macronutrient intake, cuticular hydrocarbon expression and mating success in male decorated crickets. *Journal of evolutionary biology*, 30, 711-727.

Rashed, A. & Polak, M. 2009. Does male secondary sexual trait size reveal fertilization efficiency in australian drosophila bipectinata duda (diptera: Drosophilidae)? *Biological journal of the Linnean Society,* 98**,** 406-413.

Raveh, S.; Sutalo, S.; Thonhauser, K. E.; Thoss, M.; Hettyey, A.; Winkelser, F. & Penn, D. J. 2014. Female partner preferences enhance offspring ability to survive an infection. *BMC Evolutionary Biology,* 14**,** 1-8.

Rebar, D.; Bailey, N. W. & Zuk, M. 2009. Courtship song's role during female mate choice in the field cricket teleogryllus oceanicus. *Behavioral Ecology,* 20**,** 1307-1314.

Reding, L. P.; Swaddle, J. P. & Murphy, H. A. 2013. Sexual selection hinders adaptation in experimental populations of yeast. *Biology Letters,* 9**,** 20121202.

Reinhardt, K. & Siva-Jothy, M. T. 2005. An advantage for young sperm in the house cricket acheta domesticus. *American Naturalist,* 165**,** 718-723.

Reiss, M. J. 1991. *The allometry of growth and reproduction*, Cambridge University Press.

Reynolds, J. D. & Gross, M. R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, poecilia reticulata. *Proceedings of the Royal Society B-Biological Sciences,* 250**,** 57-62.

Ringsby, T. H.; Jensen, H.; Pärn, H.; Kvalnes, T.; Boner, W.; Gillespie, R.; Holand, H.; Hagen, I. J.; Rønning, B. & Sæther, B.-E. 2015. On being the right size: Increased body size is associated with reduced telomere length under natural conditions. *Proceedings of the Royal Society B: Biological Sciences,* 282**,** 20152331.

Ripmeester, E.A.P; de Vries, A.M.; Slabbekoorn, H. 2007. Do blckbirds signal motivation to fight with their song?. Ethology, 113, 1021-1028.

Ritz, M. S. & Kohler, G. 2010. Natural and sexual selection on male behaviour and morphology, and female choice in a wild field cricket population: Spatial, temporal and analytical components. *Evolutionary Ecology,* 24**,** 985-1001.

Ritz, M. S. & Sakaluk, S. K. 2002. The role of the male's cerci in copulation and mate guarding in decorated crickets (gryllodes sigillatus). *Journal of Zoology,* 257**,** 519-523.

Rodriguez-Munoz, R.; Bretman, A. & Tregenza, T. 2011. Guarding males protect females from predation in a wild insect. *Current Biology,* 21**,** 1716-1719.

Roff, D. 1980. Optimizing development time in a seasonal environment: The 'ups and downs' of clinal variation. *Oecologia,* 45**,** 202-208.

Rost, R. & Honegger, H. W. 1987. The timing of premating and mating-behavior in a field population of the cricket gryllus-campestris l. *Behavioral Ecology and Sociobiology,* 21**,** 279-289.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proceedings of the Royal Society of London. Series B: Biological Sciences,* 263**,** 1415-1421.

Rowell, G. A. & Cade, W. H. 1993. Simulation of alternative male reproductive behaviour - calling and satellite behaviour in field crickets. *Ecological Modelling,* 65**,** 265-280.

Rundle, H. D.; Chenoweth, S. F. & Blows, M. W. 2006. The roles of natural and sexual selection during adaptation to a novel environment. *Evolution,* 60**,** 2218-2225.

Ryder, J. J. 2000. Male calling song provides a reliable signal of immune function in a cricket. *Proceedings of the Royal Society of London B: Biological Sciences,* 267**,** 1171-1175.

Ryder, J. J. & Siva-Jothy, M. T. 2000. Male calling song provides a reliable signal of immune function in a cricket. *Proceedings of the Royal Society B: Biological Sciences,* 267**,** 1171-1175.

Sadowski, J. A.; Grace, J. L. & Moore, A. J. 2002. Complex courtship behavior in the striped ground cricket, allonemobius socius (orthoptera : Gryllidae): Does social environment affect male and female behavior? *Journal of Insect Behavior,* 15**,** 69-84.

Sakaluk, S. K. 1991. Postcopulatory mate guarding in decorated crickets. *Animal Behaviour,* 41**,** 207-216.

Saleh, N. W.; Larson, E. L. & Harrison, R. G. 2014. Reproductive success and body size in the cricket gryllus firmus. *Journal of insect behavior,* 27**,** 346-356.

Savage, K. E.; Hunt, J.; Jennions, M. D. & Brooks, R. 2004. Male attractiveness covaries with fighting ability but not with prior fight outcome in house crickets. *Behavioral Ecology,* 16**,** 196-200.

Savic Veselinovic, M.; Pavkovic-Lucic, S.; Kurbalija Novicic, Z.; Jelic, M. & Andelkovic, M. 2013. Sexual selection can reduce mutational load in drosophila subobscura. *Genetika-Belgrade,* 45**,** 537-552.

Schaus, J. M. & Sakaluk, S. K. 2001. Ejaculate expenditures of male crickets in response to varying risk and intensity of sperm competition: Not all species play games. *Behavioral Ecology,* 12**,** 740-745.

Scheuber, H.; Jacot, A. & Brinkhof, M. W. G. 2003. Condition dependence of a multicomponent sexual signal in the field cricket gryllus campestris. *Animal Behaviour,* 65**,** 721-727.

Schindelin, J.; Arganda-Carreras, I.; Frise, E.; Kaynig, V.; Longair, M.; Pietzsch, T.; Preibisch, S.; Rueden, C.; Saalfeld, S.; Schmid, B.; Tinevez, J. Y.; White, D. J.; Hartenstein, V.; Eliceiri, K.; Tomancak, P. & Cardona, A. 2012. Fiji: An open-source platform for biological-image analysis. *Nature Methods,* 9**,** 676-682.

Schmidt, R.; Morrison, A.; Kunc, H.P. 2014. Sexy voices - no choices: male song in noise fails to attract females. *Animal Behaviour*, 94, 55-59.

Schuett, G. W. 1997. Body size and agonistic experience affect dominance and mating success in male copperheads. *Animal Behaviour,* 54**,** 213-224.

Schultzhaus, J. N.; Nixon, J. J.; Duran, J. A. & Carney, G. E. 2017. Diet alters drosophila melanogaster mate preference and attractiveness. *Animal Behaviour,* 123**,** 317-327.

Schwagmeyer, P. 1988. Scramble-competition polygyny in an asocial mammal: Male mobility and mating success. *The American Naturalist,* 131**,** 885-892.

Seehausen, O.; van Alphen, J.J.M.; Witte, F. 1997. Cichlid fish diversity threatened by eutrophication that curbs sexual selection. *Science*, 277, 1808-1811

Serrano-Meneses, M.; Córdoba-Aguilar, A.; Méndez, V.; Layen, S. & Székely, T. 2007. Sexual size dimorphism in the american rubyspot: Male body size predicts male competition and mating success. *Animal Behaviour,* 73**,** 987-997.

Shackleton, M. A.; Jennions, M. D. & Hunt, J. 2005. Fighting success and attractiveness as predictors of male mating success in the black field cricket, teleogryllus commodus: The effectiveness of no-choice tests. *Behavioral Ecology and Sociobiology,* 58**,** 1-8.

Shaw, K. L. 2010. Elaborate courtship enhances sperm transfer in the hawaiian swordtail cricket, laupala cerasina. *Animal Behaviour,* 79**,** 819-826.

Shaw, K. L. & Herlihy, D. P. 2000. Acoustic preference functions and song variability in the hawaiian cricket laupala cerasina. *Proceedings of the Royal Society B-Biological Sciences,* 267**,** 577-584.

Sheldon, B. C.; Merila, J.; Qvarnstrom, A.; Gustafsson, L. & Ellegren, H. 1997. Paternal genetic contribution to offspring condition predicted by size of male secondary sexual character. *Proceedings of the Royal Society B: Biological Sciences,* 264**,** 297-302.

Simmons, L. 1986a. Female choice in the field cricket gryllus bimaculatus (de geer). *Animal Behaviour,* 34**,** 1463-1470.

Simmons, L. 1986b. Inter-male competition and mating success in the field cricket, gryllus bimaculatus (de geer). *Animal Behaviour,* 34**,** 567-579.

Simmons, L. 1988. The calling song of the field cricket, gryllus bimaculatus (de geer): Constraints on transmission and its role in intermale competition and female choice. *Animal Behaviour,* 36**,** 380-394.

Simmons, L. 1992. Sexual selection and body size in a natural population of the field cricket, gryllus campestris (l.). *Journal of Orthoptera Research***,** 12-13.

Simmons, L. & Ritchie, M. 1996. Symmetry in the songs of crickets. *Proceedings of the Royal Society B: Biological Sciences,* 263**,** 1305-1311.

Simmons, L.; Tomkins, J. & Hunt, J. 1999. Sperm competition games played by dimorphic male beetles. *Proceedings of the Royal Society of London B: Biological Sciences,* 266**,** 145-150.

Simmons, L. W. 1987. Female choice contributes to offspring fitness in the field cricket, gryllus bimaculatus (de geer). *Behavioral Ecology and Sociobiology,* 21**,** 313-321.

Simmons, L. W. 1995. Correlates of male quality in the field cricket, gryllus campestris l: Age, size, and symmetry determine pairing success in field populations. *Behavioral Ecology,* 6**,** 376-381.

Simmons, L. W. & Emlen, D. J. 2006. Evolutionary trade-off between weapons and testes. *Proceedings of the National Academy of Sciences,* 103**,** 16346-16351.

Simmons, L. W.; Lüpold, S. & Fitzpatrick, J. L. 2017. Evolutionary trade-off between secondary sexual traits and ejaculates. *Trends in ecology & evolution,* 32**,** 964-976.

Simmons, L. W.; Thomas, M. L.; Simmons, F. W. & Zuk, M. 2013. Female preferences for acoustic and olfactory signals during courtship: Male crickets send multiple messages. *Behavioral Ecology,* 24**,** 1099-1107.

Simmons, L. W.; Wernham, J.; García-González, F. & Kamien, D. 2003. Variation in paternity in the field cricket teleogryllus oceanicus: No detectable influence of sperm numbers or sperm length. *Behavioral Ecology,* 14**,** 539-545.

Simmons, L. W. & Zuk, M. 1992. Variability in call structure and pairing success of male field crickets, gryllus bimaculatus - the effects of age, size and parasite load. *Animal Behaviour,* 44**,** 1145-1152.

Simpson, S.J. & Raubenheimer, D. The geometric analysis of feeding and nutrition: A user's guide. *Journal of insect physiology*, 41, 545-553

Sinervo, B.; Mendez-De-La-Cruz, F.; Miles, D. B.; Heulin, B.; Bastiaans, E.; Cruz, M. V. S.; Lara-Resendiz, R.; Martinez-Mendez, N.; Calderon-Espinosa, M. L.; Meza-Lazaro, R. N.; Gadsden, H.; Avila, L. J.; Morando, M.; De La Riva, I. J.; Sepulveda, P. V.; Rocha, C. F. D.; Ibarguengoytia, N.; Puntriano, C. A.; Massot, M.; Lepetz, V.; Oksanen, T. A.; Chapple, D. G.; Bauer, A. M.; Branch, W. R.; Clobert, J. & Sites, J. W. 2010. Erosion of lizard diversity by climate change and altered thermal niches. *Science,* 328**,** 894-899.

Sokolovska, N.; Rowe, L. & Johansson, F. 2000. Fitness and body size in mature odonates. *Ecological Entomology,* 25**,** 239-248.

Souroukis, K. & Cade, W. H. 1993. Reproductive competition and selection on male traits at varying sex-ratios in the field cricket, gryllus pennsylvanicus. *Behaviour,* 126**,** 45-62.

Souroukis, K. & Murray, A. M. 1995. Female mating behaviour in the field cricket, gryllus pennsylvanicus (orthoptera, gryllidae) at different operational sex-ratios. *Journal of Insect Behavior,* 8**,** 269-279.

Stearns, S. C. & Koella, J. C. 1986. The evolution of phenotypic plasticity in life-history traits: Predictions of reaction norms for age and size at maturity. *Evolution,* 40**,** 893-913.

Steele, R. H. & Partridge, L. 1988. A courtship advantage for small males in drosophila subobscura. *Animal Behaviour,* 36**,** 1190-1197.

Stillwell, R. C.; Moya-Laraño, J. & Fox, C. W. 2008. Selection does not favor larger body size at lower temperature in a seed-feeding beetle. *Evolution,* 62**,** 2534-2544.

Stockley, P. & Seal, N. J. 2001 Plasticity in reproductive effort of male dung flies (Scatophaga stercoraria) as a response to larval density. *Functional ecology*, 15, 96–102.

Stoffer, B. & Walker, S. E. 2012. The use of multimodal communication in mate choice decisions by female house crickets, acheta domesticus. *Animal Behaviour,* 83**,** 1131-1138.

Sullivan, B. K. 1983. Sexual selection in woodhouse's toad (bufo woodhousei). Ii. Female choice. *Animal Behaviour,* 31**,** 1011-1017.

Sullivan, B. K. & Walsberg, G. E. 1985. Call rate and aerobic capacity in woodhouse's toad (bufo woodhousei). *Herpetologica,* 41**,** 404-407.

Taborsky, M. 1998. Sperm competition in fish:Bourgeois' males and parasitic spawning. *Trends in Ecology & Evolution,* 13**,** 222-227.

Taborsky, M.; Oliveira, R. & Brockmann, H. J. 2008. The evolution of alternative reproductive tactics: Concepts and questions. *In:* TABORSKY, M., OLIVEIRA, R. & BROCKMANN, H. J. (eds.) *Alternative reproductive*

tactics: An integrative approach. Cambridge: Cambridge University Press.

Team, R. C. 2016. R: A language and environment for statistical computing. Viena, Austria: R Foundation for Statistical Computing.

Thomas, M. L.; Gray, B. & Simmons, L. W. 2011. Male crickets alter the relative expression of cuticular hydrocarbons when exposed to different acoustic environments. *Animal behaviour,* 82**,** 49-53.

Thomas, M. L. & Simmons, L. W. 2009. Male dominance influences pheromone expression, ejaculate quality, and fertilization success in the australian field cricket, teleogryllus oceanicus. *Behavioral Ecology,* 20**,** 1118-1124.

Thomas, M. L. & Simmons, L. W. 2015. No coolidge effect in the australian field cricket teleogryllus oceanicus (orthoptera: Gryllidae). *Austral Entomology,* 54**,** 433-437.

Tinghitella, R. M. 2014. Male and female crickets modulate their courtship behaviour depending on female experience with mate availability. *Animal Behaviour,* 91**,** 9-15.

Tolle, A. E. & Wagner Jr, W. E. 2011. Costly signals in a field cricket can indicate high- or low- quality direct benefits depending upon the environment. *Evolution,* 65**,** 283-294.

Travis, J. 1994. Evolution in the sailfin molly: The interplay of life-history variation and sexual selection. *In:* REAL, L. A. (ed.) *Ecological genetics.* Princeton, New Jersey: Princeton University Press.

Tregenza, T.; Simmons, L. W.; Wedell, N. & Zuk, M. 2006. Female preference for male courtship song and its role as a signal of immune function and condition. *Animal Behaviour,* 72**,** 809-818.

Tregenza, T. & Wedell, N. 1997. Definitive evidence for cuticular pheromones in a cricket. *Animal Behaviour,* 54**,** 979-984.

Tregenza, T. & Wedell, N. 1998. Benefits of multiple mates in the cricket gryllus bimaculatus. *Evolution,* 52**,** 1726-1730.

Trivers, R. 1972. Parental investment and sexual selection. *In:* CAMPBELL, B. (ed.) *Sexual selection and the descent of man 1871-1971.* Chicago: Aldine Press.

Van Breukelen, N. A. & Draud, M. 2005. The roles of male size and female eavesdropping in divorce in the monogamous convict cichlid (archocentrus nigrofasciatus, cichlidae). *Behaviour,* 142**,** 1023-1035.

Vedenina, V. Y. & Pollack, G. S. 2012. Recognition of variable courtship song in the field cricket gryllus assimilis. *Journal of Experimental Biology,* 215**,** 2210-2219.

Veen, T. & Otto, S. P. 2015. Liking the good guys: Amplifying local adaptation via the evolution of condition-dependent mate choice. *Journal of Evolutionary Biology,* 28**,** 1804-1815.

Velez, M. J. & Brockmann, H. J. 2006. Seasonal variation in female response to male calling song in the field cricket, gryllus rubens. *Ethology,* 112**,** 1041-1049.

Verburgt, L. & Ferguson, J. W. H. 2010. Mate choice in field crickets: Can females acoustically detect male body size? *Journal of Ethology,* 28**,** 141-151.

Verburgt, L.; Ferreira, M. & Ferguson, J. W. H. 2011. Male field cricket song reflects age, allowing females to prefer young males. *Animal Behaviour,* 81**,** 19-29.

Veselinovic, M. S.; Pavkovic-Lucic, S.; Novicic, Z. K.; Jelic, M.; Stamenkovic-Radak, M. & Andjelkovic, M. 2017. Mating behavior as an indicator of quality of drosophila subobscura males? *Insect Science,* 24**,** 122-132.

Vieira, M. C. & Peixoto, P. E. C. 2013. Winners and losers: A meta-analysis of functional determinants of fighting ability in arthropod contests. *Functional Ecology,* 27**,** 305-313.

Wagner Jr, W. E. 1996. Convergent song preferences between female field crickets and acoustically orienting parasitoid flies. *Behavioral Ecology,* 7**,** 279-285.

Wagner, W. E. 2005. Male field crickets that provide reproductive benefits to females incur higher costs. *Ecological Entomology,* 30**,** 350-357.

Wagner, W. E. & Basolo, A. L. 2007. The relative importance of different direct benefits in the mate choices of a field cricket. *Evolution,* 61**,** 617-622.

Wagner, W. E. & Hoback, W. W. 1999. Nutritional effects on male calling behaviour in the variable field cricket. *Animal Behaviour,* 57**,** 89-95.

Wagner, W. E.; Smeds, M. R. & Wiegmann, D. D. 2001. Experience affects female responses to male song in the variable field cricket gryllus lineaticeps (orthoptera, gryllidae). *Ethology,* 107**,** 769-776.

Walker, S. E. & Cade, W. H. 2003. The effects of temperature and age on calling song in a field cricket with a complex calling song, teleogryllus oceanicus (orthoptera: Gryllidae). *Canadian Journal of Zoology,* 81**,** 1414-1420.

Weary, D. M.; Lambrechts, M. M. & Krebs, J. R. 1991. Does singing exhaust male great tits? *Animal Behaviour,* 41**,** 540-542.

Weatherhead, P.J. & Robertson, R.J. 1979. Offspring quality and the polygyny threshold: “the sexy son hypothesis”. *American Naturalist*, 113, 201-208.

Weber, D. M.; Millar, J. S. & Neff, B. D. 2007. Male reproductive success and female preference in bushy-tailed woodrats (neotoma cinerea): Do females prefer males in good physical condition? *Canadian Journal of Zoology-Revue Canadienne De Zoologie,* 85**,** 169-176.

Wedell, N. & Tregenza, T. 1999. Successful fathers sire successful sons. *Evolution,* 53**,** 620-625.

Welch, A. M.; Semlitsch, R. D. & Gerhardt, H. C. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. *Science,* 280**,** 1928-1930.

Werner, E. E. & Anholt, B. R. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. *The American Naturalist,* 142**,** 242-272.

Whattam, E. M. & Bertram, S. M. 2011. Effects of juvenile and adult condition on long-distance call components in the jamaican field cricket, gryllus assimilis. *Animal Behaviour,* 81**,** 135-144.

Wong, B.; Candolin, U.; Lindström, K. 2007. Environmental deterioration compromises socially enforced signals of male quality in three-spined sticklebacks. *The American Naturalist*, 170, 184-189.

Wright, D.; Nakamichi, R.; Krause, J. & Butlin, R. K. 2006. Qtl analysis of behavioral and morphological differentiation between wild and laboratory zebrafish (danio rerio). *Behavior genetics,* 36**,** 271-284.

Wynn, H. & Vahed, K. 2004. Male gryllus bimaculatus guard females to delay them from mating with rival males and to obtain repeated copulations. *Journal of Insect Behavior,* 17**,** 53-66.

Zahavi, A. 1975. Mate selection - selection for a handicap. *Journal of Theoretical Biology,* 53**,** 205-214.

Zajitschek, F.; Hunt, J.; Jennions, M.D.; Hall, M.D.; Brooks, R.C. 2009. Effects of juvenile and adult diet on ageing and reproductive effort of male and female black field crickets, *Teleogryllus commodus*. *Functional Ecology*, 23, 302-311.

Zhemchuzhnikov, M. K.; Kutcherov, D. A.; Kymre, J. H. & Knyazev, A. N. 2017. Louder songs can enhance attractiveness of old male crickets (gryllus bimaculatus). *Journal of Insect Behavior,* 30**,** 211-219.

Zhu, B.; Wang, J.; Zhao, L.; Sun, Z.; Brauth, S. E.; Tang, Y. & Cui, J. 2016. Bigger is not always better: Females prefer males of mean body size in philautus odontotarsus. *PloS one,* 11**,** e0149879.

Zuk, M. 1988. Parasite load, body size, and age of wild-caught male field crickets (orthoptera, gryllidae) - effects of sexual selection. *Evolution,* 42**,** 969-976.

Zuk, M. & Kolluru, G. 1998. Exploitation of sexual signals by predators and parasitoids. *The Quarterly Review of Biology*, 73, 415-438.

Zuk, M.; Rotenberry, J. T. & Tinghitella, R. M. 2006. Silent night: Adaptive disappearance of a sexual signal in a parasitized population of field crickets. *Biology letters,* 2**,** 521-524.

Zuk, M.; Thornhill, R.; Ligon, J. D. & Johnson, K. 1990. Parasites and mate choice in red jungle fowl. *American Zoologist,* 30**,** 235-244.